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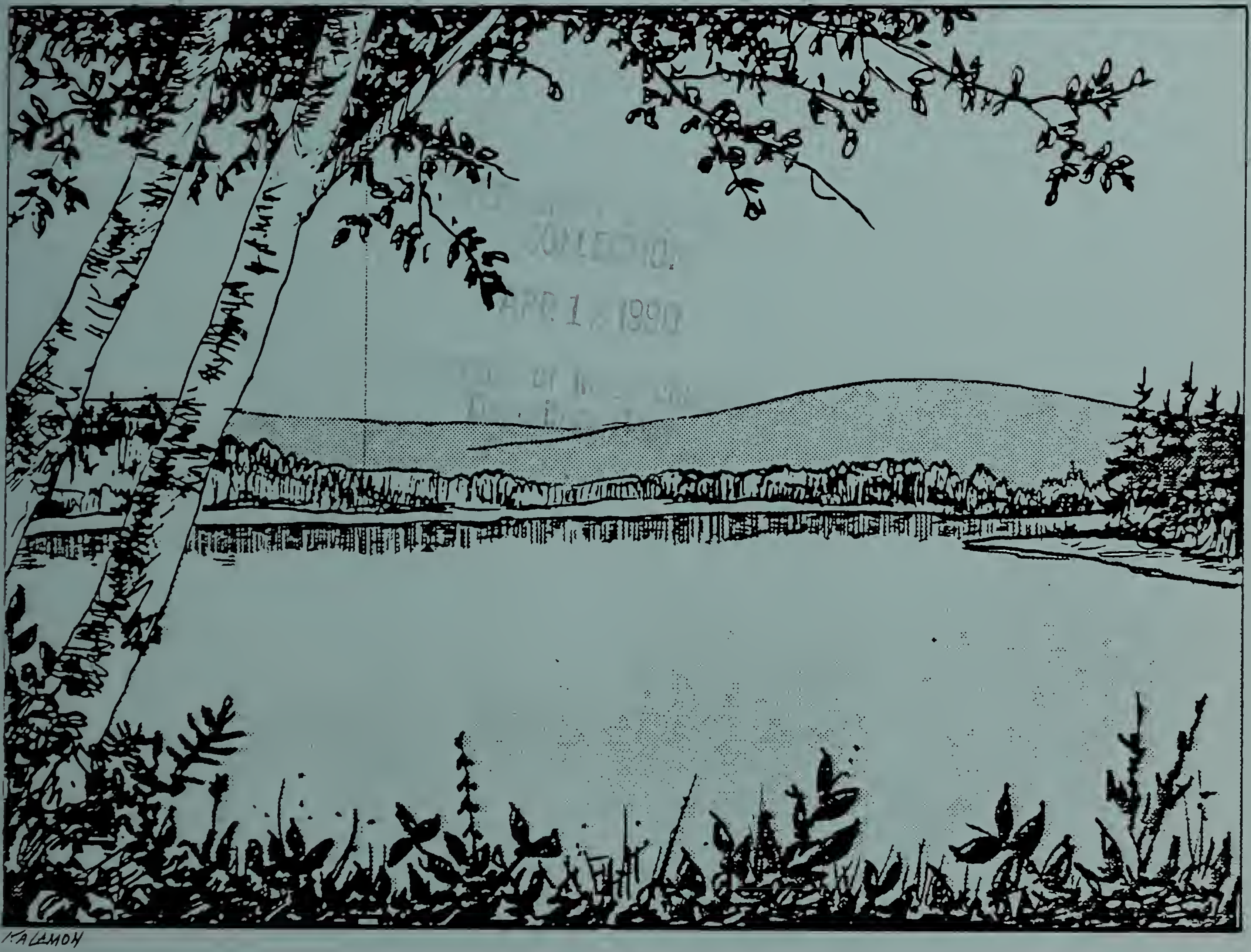
An Examination of Die-off of Fecal Coliform and Fecal Streptococci Bacteria in the Ware River

1985



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AN EXAMINATION OF DIE-OFF OF FECAL COLIFORM AND FECAL STREPTOCOCCI BACTERIA IN THE WARE RIVER

1985

BY

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DIVISION OF WATER POLLUTION CONTROL
CORNELIUS J. O'LEARY, ACTING DIRECTOR

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INTRODUCTION

Fecal coliform bacteria is the indicator of fecal contamination typically used to assess bacterial quality of recreational waters in the United States. In recent years some attention has been given to other indicator organisms such as *Escherichia coli* and enterococci bacteria (U.S. EPA, 1986). Interest in these indicators has resulted in part from studies which have shown that fecal coliform bacteria can multiply in natural waters (Kittrell and Furfari, 1963). This reduces their effectiveness as an indicator organism because the standards are based on ratios of indicator bacteria to bacterial pathogens which would no longer be viable if growth occurred. Viruses cannot multiply outside of their host cells, nor is there any known ratio of indicator organisms to viral particles.

Fecal coliform bacteria have also exhibited a phenomenon known as regrowth. This occurs where bacteria are treated with chlorine to reduce their numbers, usually in a sewage treatment plant. However, instead of killing the bacteria, they are only "stressed." This can result from inadequate contact time or a chlorine residual that is too low. Although these stressed organisms do not appear in the bacterial counts in samples taken after the chlorine contact chamber, a few hours after discharge they are again viable and can produce the distinguishing gas or colony characteristics required for identification.

Not all investigators have found these multiplication or regrowth phenomena (Deaner and Kerri, 1969) instead, their studies have shown a typical die-off curve for fecal coliform bacteria below a point or nonpoint source of fecal contamination. Die-off of fecal coliform bacteria can result from ultraviolet light, elevated water temperatures, pH values greater than 8.5 or less than 5.5 standard units, and predation by invertebrates.

In 1985, a mini-study was conducted on the Ware River to examine which of these phenomena may occur in the fresh water and flowing waters of Massachusetts. A six-mile section of the Ware River below the Ware Wastewater Treatment Plant (WWTP) was chosen for study. Below the sewage treatment plant there are few tributaries and no other known sources of fecal contamination. At the Ware Treatment Plant chlorination is the method of disinfection used to meet the current discharge standard of 200 fecal coliform/100ml and a chlorine residual limit of 1 ppm. Samples were collected for fecal streptococci bacteria, as well as fecal coliform bacteria, which offered a comparison of growth or die-off rates. Fecal streptococci bacteria are believed to survive longer than fecal coliform bacteria in natural waters and are not believed to multiply in natural waters (Clausen et al., 1977).

Several assumptions were made and several problems occurred during the sampling period.

The assumptions were:

1) Incoming tributaries do not contribute significantly to the flow or to the bacterial counts so that either dilution effects or increases in bacterial counts are not observed below the tributaries;

2) the chlorine concentration would be altered on Friday and maintained at a relatively constant level over the weekend.

The problems included:

1) Difficulties at the Ware Wastewater Treatment Plant in maintaining the chlorination level desired;
2) short travel time from above the Ware WWTP, Ware to Forest Lake Road in Palmer;
3) low bacterial contributions discharged from the plant; and
4) high instream bacterial levels.

In addition to the regrowth/die-off study, sampling stations were also established directly below the Ware Wastewater Treatment Plant to determine if fecal coliform counts increase within the riffle zone. This could result from the physical break-up of solids resulting in exposure of bacteria that had not been isolated before. A grid of sampling stations was established to examine if this does occur.

SAMPLING DESIGN

Die-Off/Regrowth Study

During the summer of 1985 bacterial samples were collected at the Ware Wastewater Treatment plant from the chlorine contact chamber just before discharge and also at the discharge pipe where it enters the Ware River. Stations were also sampled at approximately one-mile intervals downstream from the plant for a total of six miles (See Table 1, Figure 1). Samples were collected on July 1, 8, 10, 17, 24, 29, 31 and August 5 and 7. Three days prior to sampling, personnel at the treatment plant were requested to alter the chlorination regime so that "steady state" conditions could be established. A schedule was submitted to the plant operators so that one of three chlorination regimes was chosen each week. These levels were meant to be:

high - approximately 0.7-1.0 mg/l total chlorine residual
medium - approximately 0.4-0.6 mg/l total chlorine residual
low - approximately 0.0-0.3 mg/l total chlorine residual

The requested chlorination regimes and the actual chlorine residuals measured at the chlorine contact chamber are presented in Table 2.

Riffle Zone Study

During the last four weeks of the study samples were also collected in the riffle zone below the treatment plant discharge. The riffle zone samples were collected in 50-foot increments starting at the discharge pipe. The sampling stations extended 210 feet downstream in the river to a point in a pool area just below the riffle zone. Sampling stations also extended in transects across the river (see Figure 2). Station numbers were assigned so that the numbers on the right-hand side of the river, facing downstream reflect the distance from the discharge pipe, i.e., 50, 100, 150, 210 respectively. The transect station numbers are in numerical order across, i.e., 51, 52, 53.

TABLE 1

LOCATIONS OF SAMPLING STATIONS

WARE RIVER

1985

STATION NUMBER	LOCATION DESCRIPTION	MILE POINT
1	Approximately 15 feet upstream from the Ware Wastewater Treatment Plant, Ware. Discharge sampled in middle of the river.	10.8
2	At the discharge pipe of the chlorine contact chamber, Ware Wastewater Treatment Plant, Ware	10.8
3	Approximately 15-feet downstream from the treatment plant discharge, collected along the western shore, Ware	10.8
4	Gibbs Crossing USGS Gaging Station, Ware	9.1
5	Old Belchertown Road, near Route 32, Ware	8.1
5a	Unnamed Brook, located approximately 20-feet above Station 5, Ware	0.0, 8.1
6	Behind Metropolitan Airport, Palmer	6.3
7	At Camp Ramoit, on east side of the river, Palmer	5.2
8	At Forest Lake Road, off Emery St., Palmer	4.8

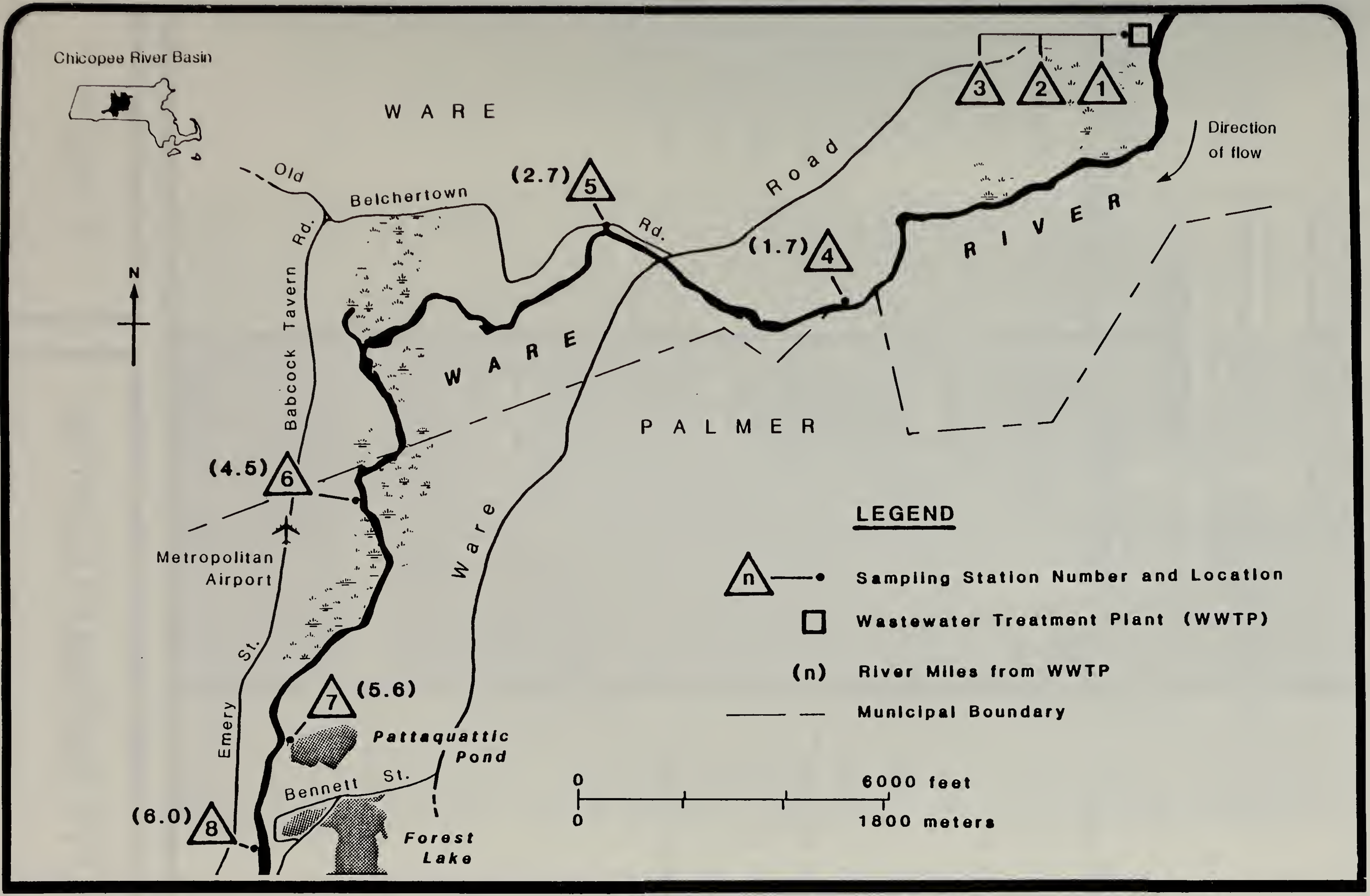


Figure 1

LOCATION OF SAMPLING STATIONS

TABLE 2

TOTAL CHLORINE RESIDUAL MEASURED AT THE CHLORINE CONTACT CHAMBER AND REQUESTED CHLORINE RESIDUAL

WARE WASTEWATER TREATMENT PLANT

DATE	REQUESTED CHLORINE RESIDUAL	MEASURED TOTAL CHLORINE RESIDUAL(ppm)
July 1, 1985	high (0.7-1.0 ppm)	0.35
July 8, 1985	high	1.00
July 10, 1985	none (0.0 ppm)	0.02
July 17, 1985	medium (0.4-0.6 ppm)	0.90
July 24, 1985	none	0.05
July 29, 1985	low (0.0-0.3 ppm)	0.55
July 31, 1985	low	0.85
August 5, 1985	none	0.10
August 7, 1985	low	0.30

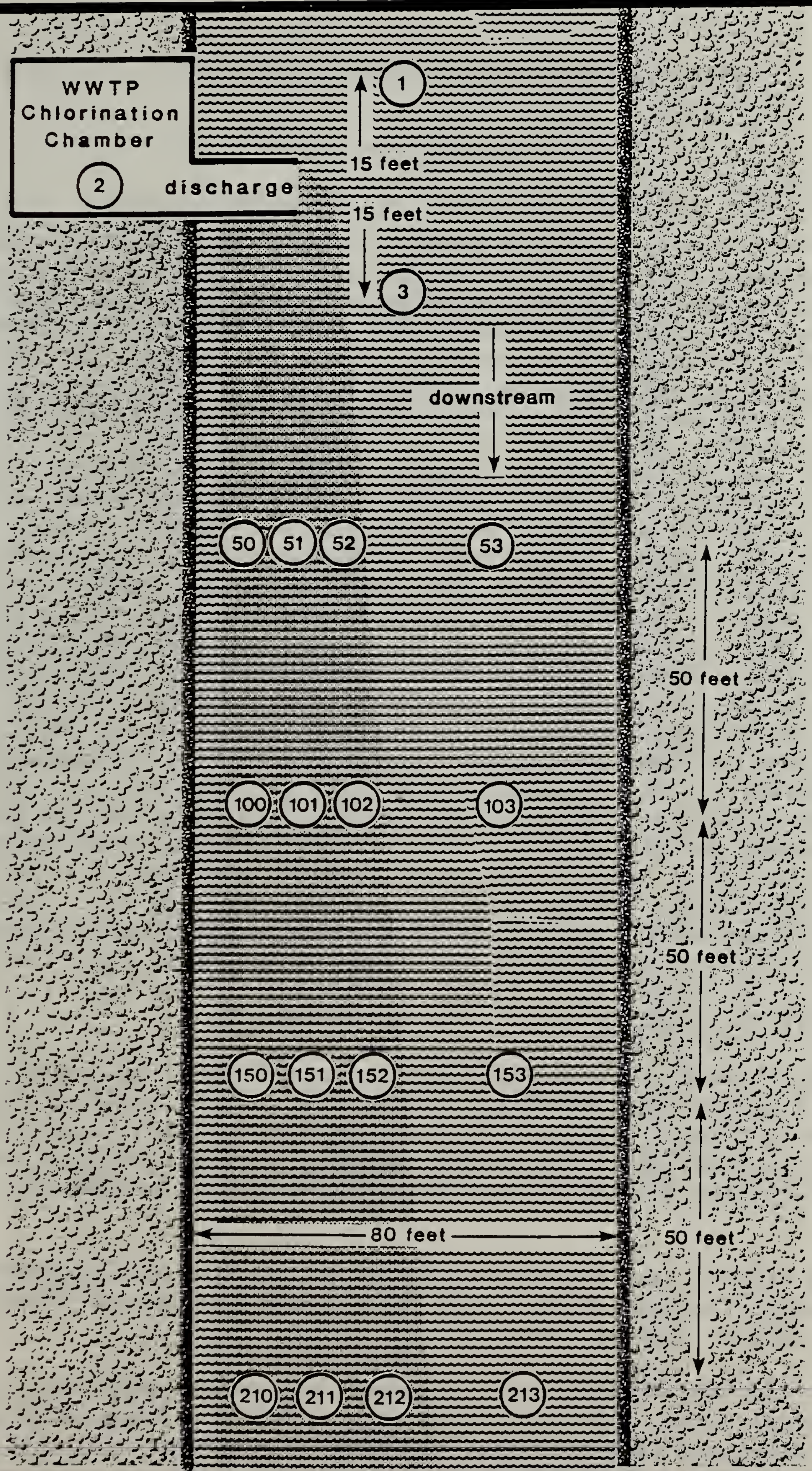


Figure 2

INSTREAM SAMPLING STATIONS

MATERIALS AND METHODS: DIE-OFF STUDY AND RIFFLE ZONE STUDY

Field Methods

Water samples were collected from the Ware River in western Massachusetts during the summer of 1985. Grab samples for bacterial analysis were collected at stations approximately 15 feet upstream and downstream of the outfall of the Ware Wastewater Treatment Plant and at six locations further downstream, including one unnamed tributary (see Table 1, Figure 1). Samples were collected by wading into the river and following standard bacterial collection procedures (American Public Health Association, 1985). Samples were also taken from the chlorine contact chamber at the Ware Wastewater Treatment Plant. At this station the samples were collected with a bucket at the end of the chlorine contact chamber. At each station triplicate bacterial samples were collected. The samples were collected in 200 ml sterile screw-cap bottles (American Public Health Association, 1985). In addition, bottles used for collection at the treatment plant and directly downstream from the plant were treated with sodium thiosulfate to quench the residual chlorine (American Public Health Association, 1985). All the bottles used in the Riffle Zone Study were treated with sodium thiosulfate. However, only single grab samples were collected by wading into the river at each location. Samples were collected at downstream locations first, which was followed by the next set of upstream stations. This was done to avoid disturbing the sediments which could potentially be resuspended and contaminate the samples. Also, no samples were collected at a station until it was visually determined that any sediments disturbed by the collector had resettled or had been washed downstream. Standard bacterial collection procedures were followed (American Public Health Association, 1985). Immediately after collection, the samples from both studies were labeled and stored on ice. Samples were brought to the Department of Environmental Quality Engineering's laboratory (Lawrence Experiment Station) for analysis within six hours of their collection.

Physical Measurements

At each in-stream station included in the die-off study water temperature and light intensity were measured. Temperature was measured in centigrade with a hand-held alcohol thermometer (Fisherbrand 15-921B). A General Electric triple range 214 light meter was used to measure the light intensity at the sample location. The light meter was held just above the surface of the water (see Table 3).

A HACH kit was used to measure total and free chlorine residual at the Ware Wastewater Treatment Plant. The DPD colorimetric method (American Public Health Association, 1985) was used.

At one station, Gibbs Crossing (Station 4), measurements from the U.S. Geological Survey flow staff gage were recorded on each sampling day. A rating table developed from this gage was used to determine discharge.

The study segment was divided into two reaches based on hydrological characteristics. Reach 1 represented 2.7 miles and extended from the Ware WWTP to Gibbs Crossing. It included Stations 1-5. Reach 2, 3.3 miles, extended from Gibbs Crossing to State Street and included sampling Stations 6-8. Reach 2 was

TABLE 3

WARE RIVER SAMPLING

WATER TEMPERATURE AND LIGHT METER READINGS

DATE	STATION NUMBER							
	1	2	3	4	5	6	7	8
7/1/85	20(1) --(2)	-- --	19.5 --	20 --	19 --	20 --	19 --	19 --
7/8/85	23 9000	-- --	22.5 4000	22 1000	22 9500	22 7500	22 500	22.5 8800
7/10/85	25 7000	-- --	25 7000	24 1500	23.5 2300	24 4500	24 1500	24.5 6500
7/17/85	24 8000	-- --	24 8000	23.5 4000	23.5 8600	23 5000	23.5 200	23.5 8600
7/24/85	23 6900	21 --	23 6900	22 625	22 7250	22 7500	21 4250	22 7250
7/29/85	24 4200	-- --	24 4200	23 7000	23.5 8800	22.5 6500	24 480	24.5 6800
7/31/85	23 2750	21 --	23 2750	22 775	22 725	22.5 250	24 250	24.5 75
8/5/85	23 6000	20.5 --	23 6000	23 7100	23.5 8200	22.5 3250	23.5 430	23.5 6900
8/7/85	22.5 2800	21.5 -	22.5 2800	22 1700	22 1700	22 800	22 1300	22.5 1850

(1)Temperature = °C
(2)Lightmeter Reading = Foot Candles

0.6 miles longer than Reach 1, yet time-of-travel through Reach 2 was four times longer due to the presence of the Diamond International Impoundment situated less than one mile downstream at Thorndike Village. The time-of-travel studies done by personnel from the Division of Water Pollution Control/Technical Services Branch (Kimball, 1972, 1973, 1980) provided the information necessary to determine flow velocities at different discharge rates. This information was used in the calculation of the die-off rates of the fecal coliform bacteria.

The die-off formula used is as follows (Camp Dresser and McKee, 1980):

$$KF = \frac{2.303}{X1-X2} \quad V \log \frac{F1}{F2}$$

where: KF = 1/day, base e
V = stream velocity in feet per second
F1, F2 - fecal coliform densities (cfu/100 ml) at X1 and X2, respectively
X1, X2 - two spatial locations (i.e., upstream-downstream stations)

Bacteriological Analyses

Three bacterial indicators were used in the die-off study: fecal coliform bacteria, fecal streptococci bacteria and enterococci bacteria. Enterococci data were only occasionally collected on an experimental basis using mE medium. These data were not included in the analysis but are presented in the appendix. The membrane filter procedure was used for each of these indicators. Results are recorded as colony forming units per 100 ml (CFU/100 ml). In the presentation of the bacterial data, "less-than" values were considered to be one-half of the expressed value (e.g., <10 would be 5) for graphical and calculation purposes.

DIFCO M-FC media and KF media were used for the fecal coliform and fecal streptococci bacteria, respectively. The enterococci medium was prepared as described by Dufour (1980).

Only fecal coliform and fecal streptococci were sampled in the riffle zone study.

RESULTS OF THE DIE-OFF/REGROWTH STUDY

Several general statements can be made concerning the results of the Ware River die-off study. The KF values, which were calculated for the two major reaches included in the study, indicate that the overall average die-off rate of the fecal coliform bacteria is twice as fast as that of the fecal streptococci bacteria (1.21 day^{-1} to 0.61 day^{-1}, respectively).

The typical ranges of KF for fecal coliform bacteria as presented by Camp Dresser and McKee, Inc. (1980), were 0.5-3.5 per day with most values between 1.0 and 2.5 per day. The Ware River KF values for the fecal coliform bacteria ranged from -2.87 to 5.47; for the fecal streptococci it was -2.38 to 3.91. Tables 5-6 list the KF values calculated for Reach 1 and Reach 2 on each sampling date. Figures 3 to 11 depict changes in the fecal coliform and fecal streptococci counts versus river miles and are described in further detail in the following sections. Table 4 presents the flow data used in the calculations of KF.

In Reach 2 there were four occasions out of nine when the fecal coliform bacterial counts increased rather than decreased over Stations 4 to 8. This is shown by the negative KF rates. This occurred once in Reach 1 for the fecal coliform bacteria.

In Reach 2 there was only one occasion out of nine when the fecal streptococci bacterial counts increased rather than decreased. On this same date, July 17, 1985, the fecal coliform bacteria in Reach 2 also increased. This situation indicates the possibility of an in-stream source on this date. The fecal streptococci bacteria are not known to multiply in the environment.

Examination of graphs from the die-off study typically showed a slight increase by Station 7 which perhaps indicates multiplication or regrowth of the bacteria. However, evidence of exponential growth typically did not occur. The short travel time between Station 2 and Station 8 may not have provided enough time for the enzyme systems of the stressed bacteria to recover. Also, it is possible that the water hardness may affect these results. Apparently, soft water (or very hard water) may be toxic to fecal coliform bacteria to some degree while moderately hard to hard waters may aid in their multiplication (Kenner, 1982). The hardness of the Ware River would be considered low (soft water). Sampling done by the Division of Water Pollution Control, Technical Services Branch found values that ranged from 11-21 mg/l (MA Division of Water Pollution Control, 1986).

The instream die-off patterns often exhibited an initial drastic decrease through the first couple of stations, followed by a more gradual decrease, and sometimes even an increase, further downstream.

Samples at Station 1 (instream station) were collected just upstream of the treatment plant discharge and often had counts that were higher than Station 2 (the treatment plant discharge sampled at the end of the chlorination chamber). In many ways the elevated bacterial densities already present in the Ware River when it reaches the Ware Wastewater Treatment Plant can be confounding because it is not known how long these bacteria have been in the water or if they have been chlorinated or stressed in other ways. Nor is it known if they were in a lag phase of growth or nearing exponential growth. This too could affect

TABLE 4

WARE RIVER SAMPLING

FLOW, TIME OF TRAVEL, CHLORINE RESIDUAL*, PRECIPITATION

DATE	FLOW(cfs)	T.O.T.(hrs.) Reach 1	T.O.T.(hrs.) Reach 2	TOTAL CHLORINE(mg/l)	PRECIPITATION(inch
7/1/85	24.9	11	41	0.35	0.58
7/8/85	52.7	7.3	24	1.00	0.12
7/10/85	73.0	6.2	19.5	0.02	0.06
7/17/85	205.0	3.6	9.5	0.90	0.99
7/24/85	36.9	8.7	31	0.05	0.40
7/29/85	73.0	6.2	19.5	0.55	0.72
7/31/85	36.9	8.7	31	0.85	0.00
8/5/85	52.7	7.3	24	0.10	0.00
8/7/85	36.9	8.7	31	0.30	0.00

T.O.T. = Time of Travel from WWTP to Station #8
Precipitation = Amount of rainfall in previous three days including day of sampling as measured at the Ware WWTP by treatment plant personnel.
*Chlorine residual of the treatment plant effluent.

TABLE 5

WARE RIVER SAMPLING

DIE-OFF RATES CALCULATED AS

KF FOR FECAL COLIFORM BACTERIA (day^{-1})

DATE	KF(Reach 1)	KF(Reach 2)	FLOW(cfs)[1]
7/1/85	+0.89	-1.60	24.9
7/8/85	+3.75	-0.06	52.7
7/10/85	-1.88	+1.57	73.0
7/17/85	+5.47	-2.87	205.0
7/24/85	+2.28	+0.91	36.9
7/29/85	+2.87	-0.22	73.0
7/31/85	+4.66	+0.74	36.9
8/5/85	+3.24	+0.11	52.7
8/7/85	+1.54	+0.53	36.9

---

[1] USGS Gage at Gibbs Crossing

TABLE 6

WARE RIVER SAMPLING

DIE-OFF RATES CALCULATED AS

KF FOR FECAL STREPTOCOCCI BACTERIA (day^{-1})

DATE	KF(Reach 1)	KF(Reach 2)	FLOW(cfs)[1]
7/1/85	+1.98	+0.28	24.9
7/8/85	+3.91	+0.73	52.7
7/10/85	-1.53	+0.98	73.0
7/17/85	0	-2.38	205.0
7/24/85	0	+1.00	36.9
7/29/85	+1.84	+0.17	73.0
7/31/85	+0.38	+0.76	36.9
8/5/85	+0.76	+0.98	52.7
8/7/85	+0.52	+0.60	36.9

---

[1] USGS Gage at Gibbs Crossing

results downstream. In this study the bacteria contributed upstream were the only bacteria available for observation as a result of low bacterial counts contributed by the Ware Wastewater Treatment Plant. This effect can be noted on the graphs by the typical drop in the counts at Station 2. Only on July 10 and August 5 were the effluent bacterial counts higher than the in-stream counts. Thus, this combination of chlorinated and non-chlorinated bacteria was the population under investigation.

Results and Discussion

July 1 - The fecal coliform CFU counts decreased over the six miles, although the last station showed a slight increase. The largest drop was seen over the first two miles. The fecal streptococci showed a slower rate of decrease. However, at the last station the count dropped rapidly while the fecal coliform data showed an increase. Even though a high chlorination regime was requested on this date yet the total chlorine residual was only 0.35 mg/l; the fecal coliform count was 42 CFU/100ml (see Figure 3).

July 8 - The fecal coliform count dropped rapidly over the first two miles as did the fecal streptococci. Fecal coliform at the last station showed a slight increase while fecal streptococci a larger decrease. A high chlorination regime was requested on this date and the chlorine residual was measured at 1.0 mg/l. The fecal coliform count at Station 2 was 5 CFU/100 ml (see Figure 4).

July 10 - There was a slight increase in fecal coliform and fecal streptococci over the first two miles. The die-off rates of both bacterial groups leveled off until river-mile six where the fecal coliform continued to decline at a slightly higher rate than that of the fecal streptococci bacteria. On this date no chlorination was requested. The chlorine residual was low 0.02 mg/l and the fecal coliform count at the chlorine contact chamber was the highest (16000 CFU/100 ml) of any of the sampling counts (see Figure 5).

July 17 - The fecal coliform counts followed no discernible pattern on this date. It is likely that fecal coliform bacteria were contributed to the Ware River by nonpoint sources of fecal contamination. This runoff resulted from 0.99 inches of precipitation which fell during the previous evening. It is possible that some multiplication of the bacteria occurred over the first two miles, but the increase in the fecal streptococci over this same distance reduces this possibility because the fecal streptococci are not known to multiply in the natural environment. Although a moderate chlorination regime was requested, a total chlorine residual of 0.90 mg/l was recorded at the end of the chlorine contact chamber. The fecal coliform count was again low (40 CFU/100 ml) (see Figure 6).

July 24 - On this day the total residual chlorine was 0.05 mg/l at the chlorine contact chamber and high bacterial counts were expected. Instead, at Station 2 the fecal coliform mean was only 21/100 ml. Why this occurred at the plant is not known. Instream fecal coliform counts showed rapid die-off over the first two miles and then continued to decrease over the remaining four. The fecal streptococci fluctuated over this distance (See Figure 7).

July 29 - The fecal coliform counts on this date were highly erratic. Again the total chlorine residual was moderate (0.55 mg/l) yet the mean of the fecal coliform as Station 2 was only 7/100 ml. Over the first two miles the instream fecal coliform counts decreased rapidly and then increased over the following

Figure 3

WARE RIVER BACTERIOLOGICAL DATA

JULY 1,1985

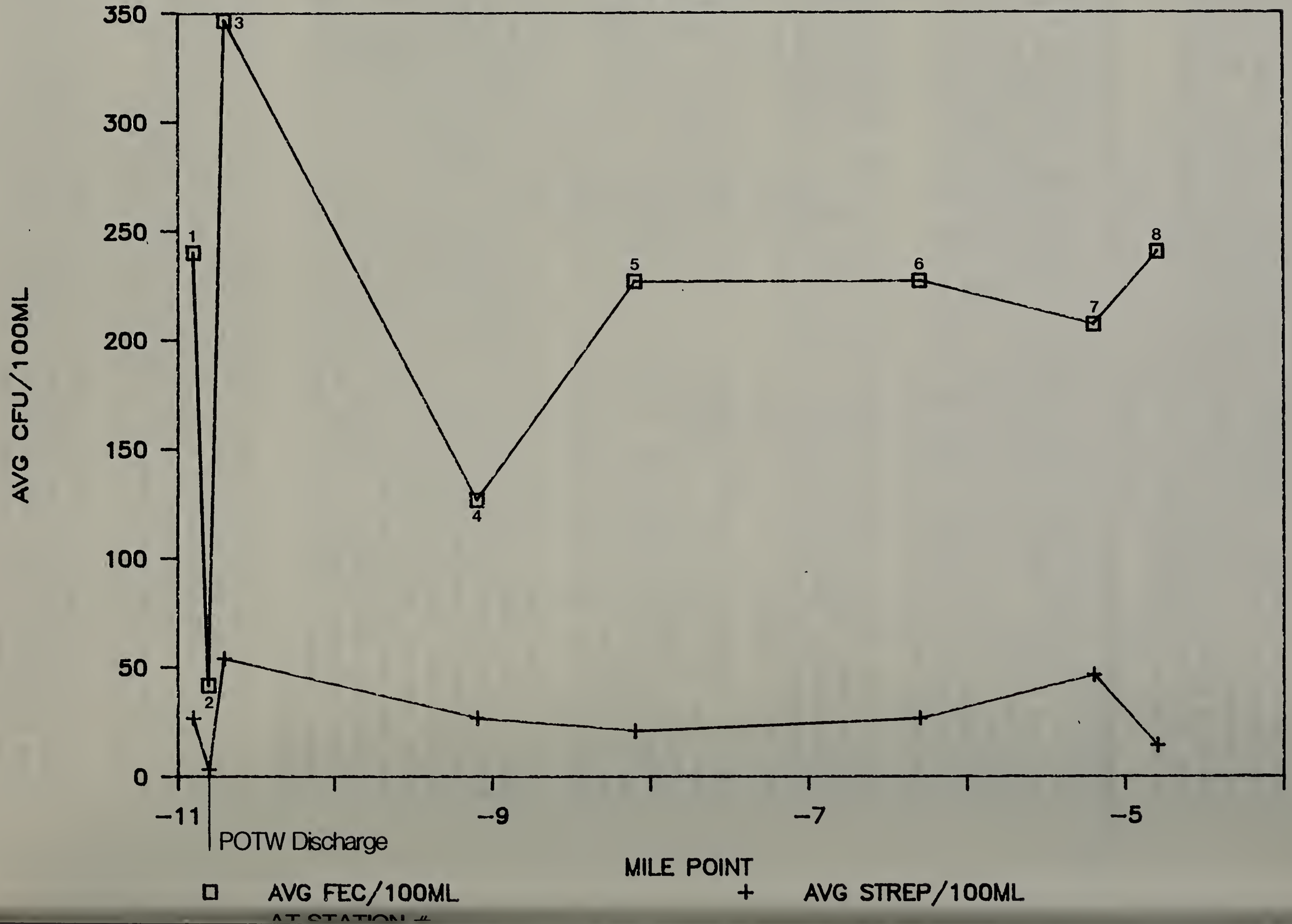


Figure 4

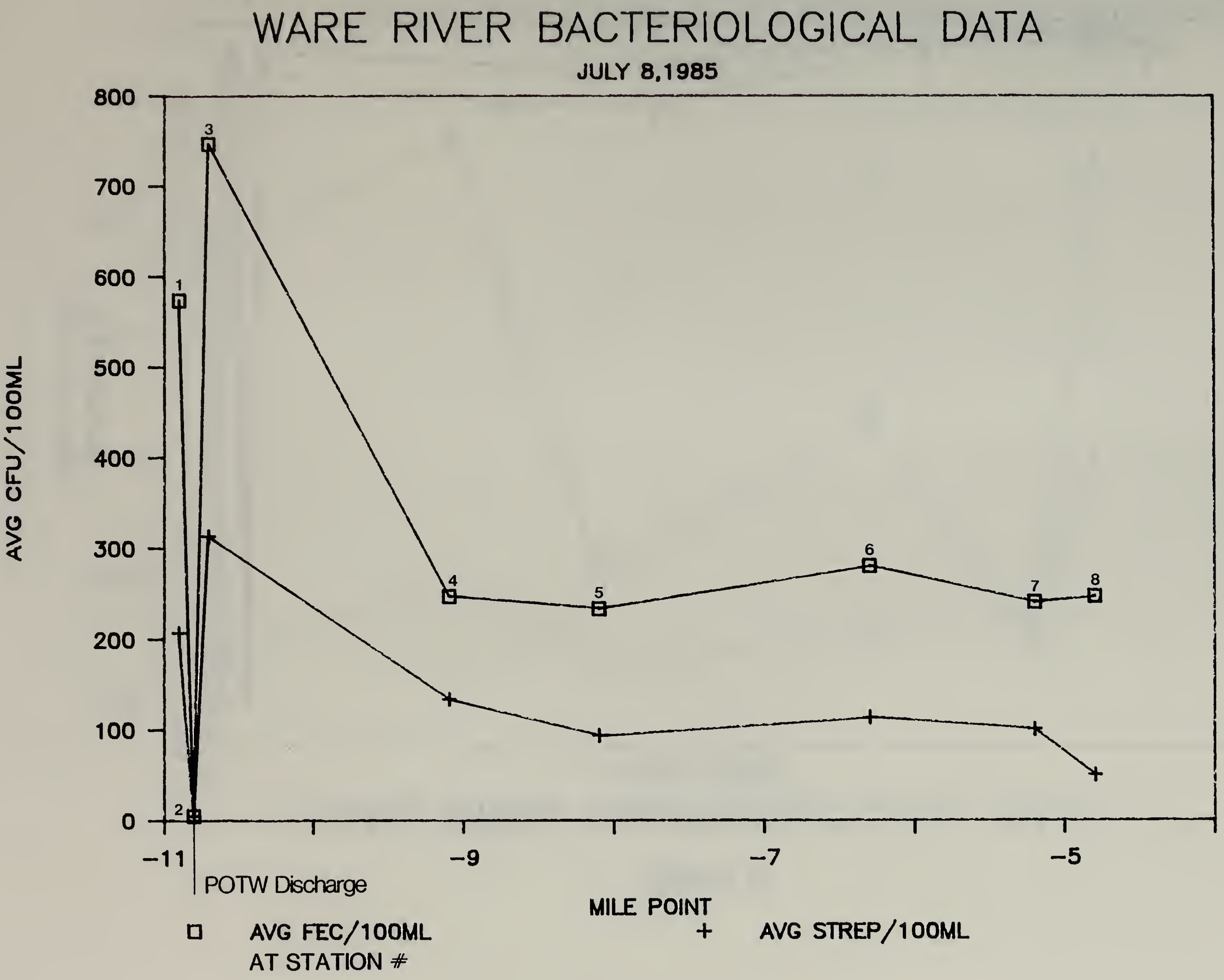

WARE RIVER BACTERIOLOGICAL DATA
JULY 8,1985
AVG CFU/100ML
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
−11
−9
−7
−5
POTW Discharge
MILE POINT
□ AVG FEC/100ML
AT STATION #
+ AVG STREP/100ML

Figure 5

WARE RIVER BACTERIOLOGICAL DATA

JULY 10,1985

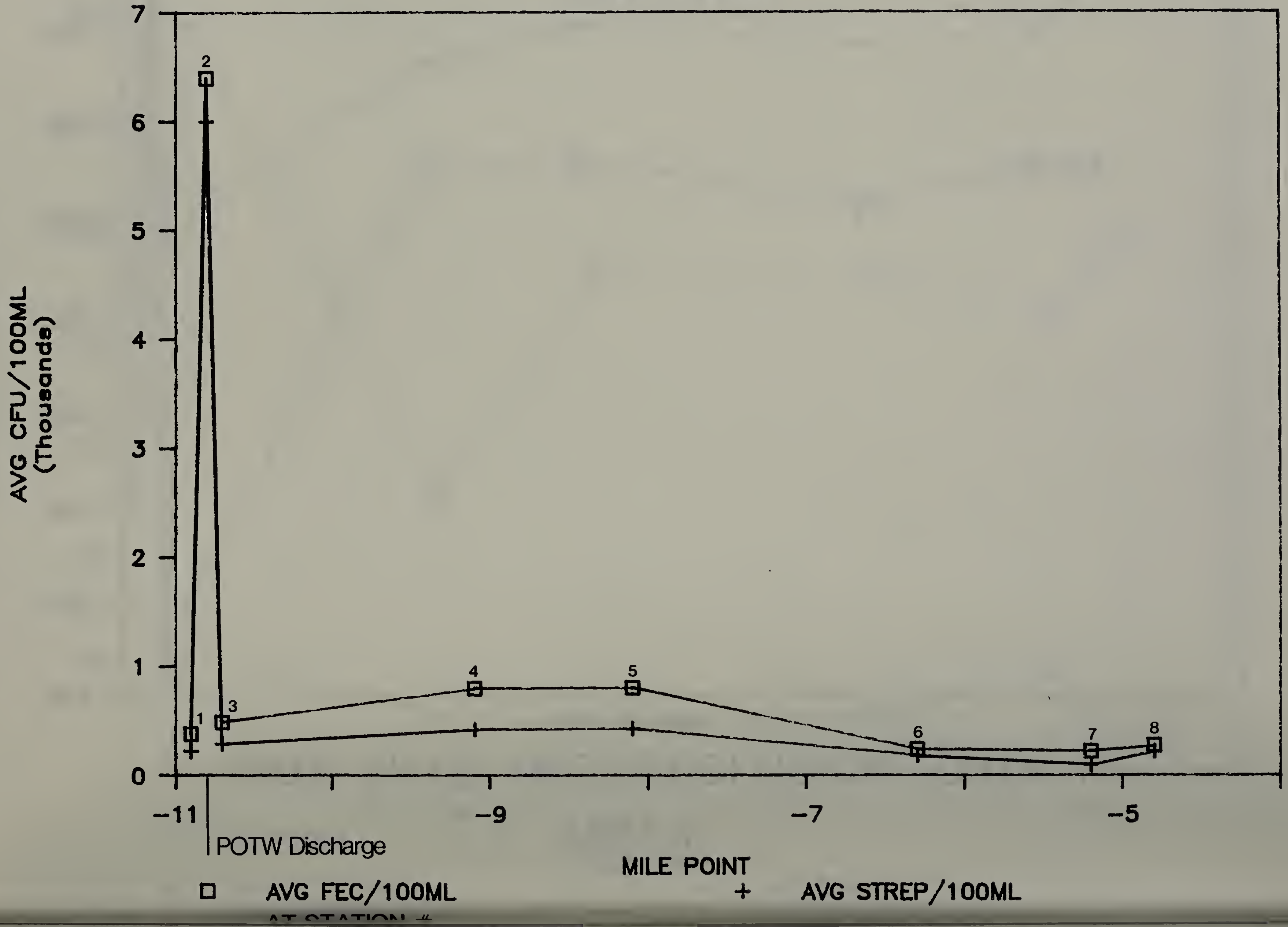


Figure 6

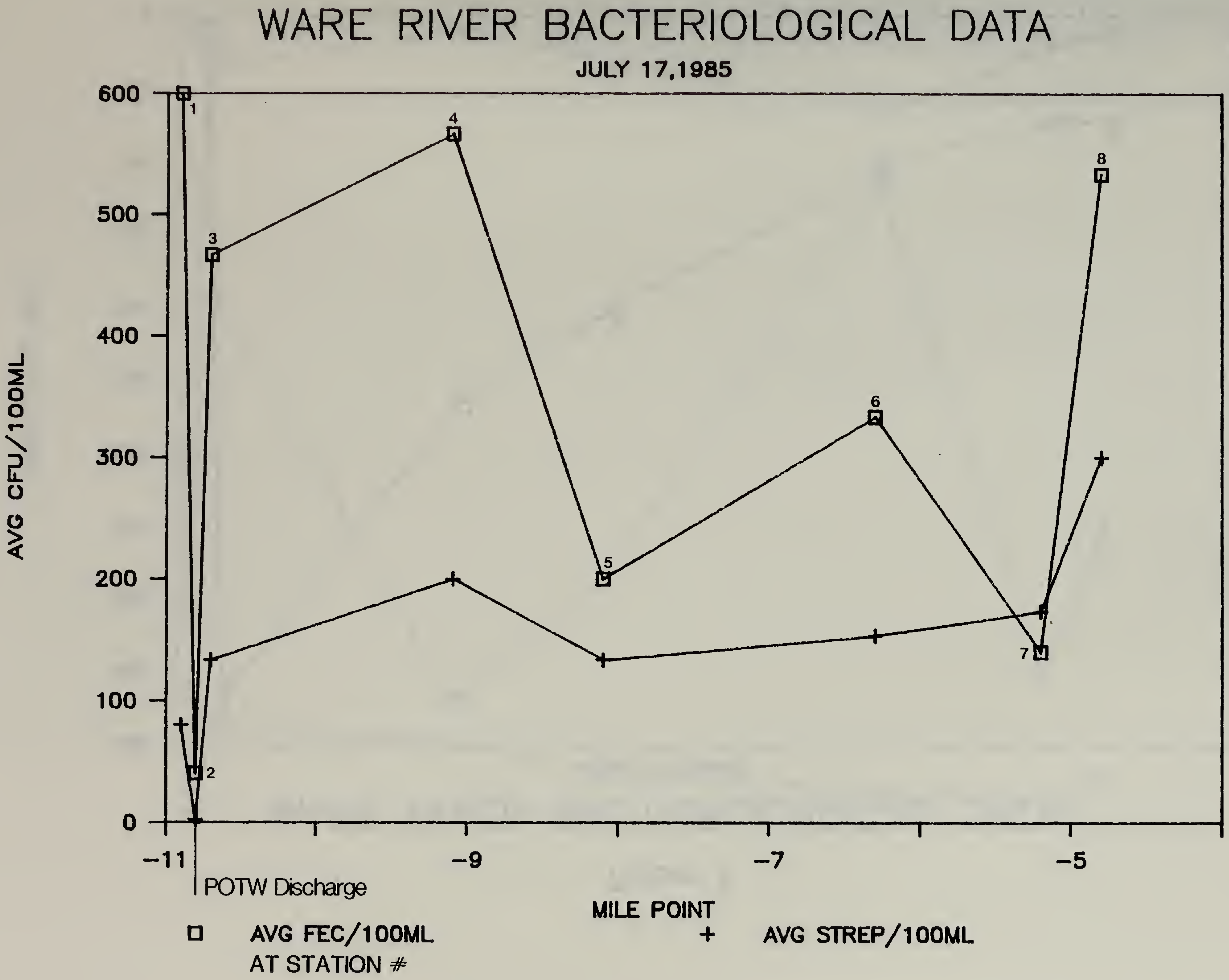

WARE RIVER BACTERIOLOGICAL DATA
JULY 17,1985
AVG CFU/100ML
600
500
400
300
200
100
0
-11
-9
-7
-5
MILE POINT
POTW Discharge
1
2
3
4
5
6
7
8
□ AVG FEC/100ML AT STATION #
+ AVG STREP/100ML

Figure 7

WARE RIVER BACTERIOLOGICAL DATA

JULY 24,1985

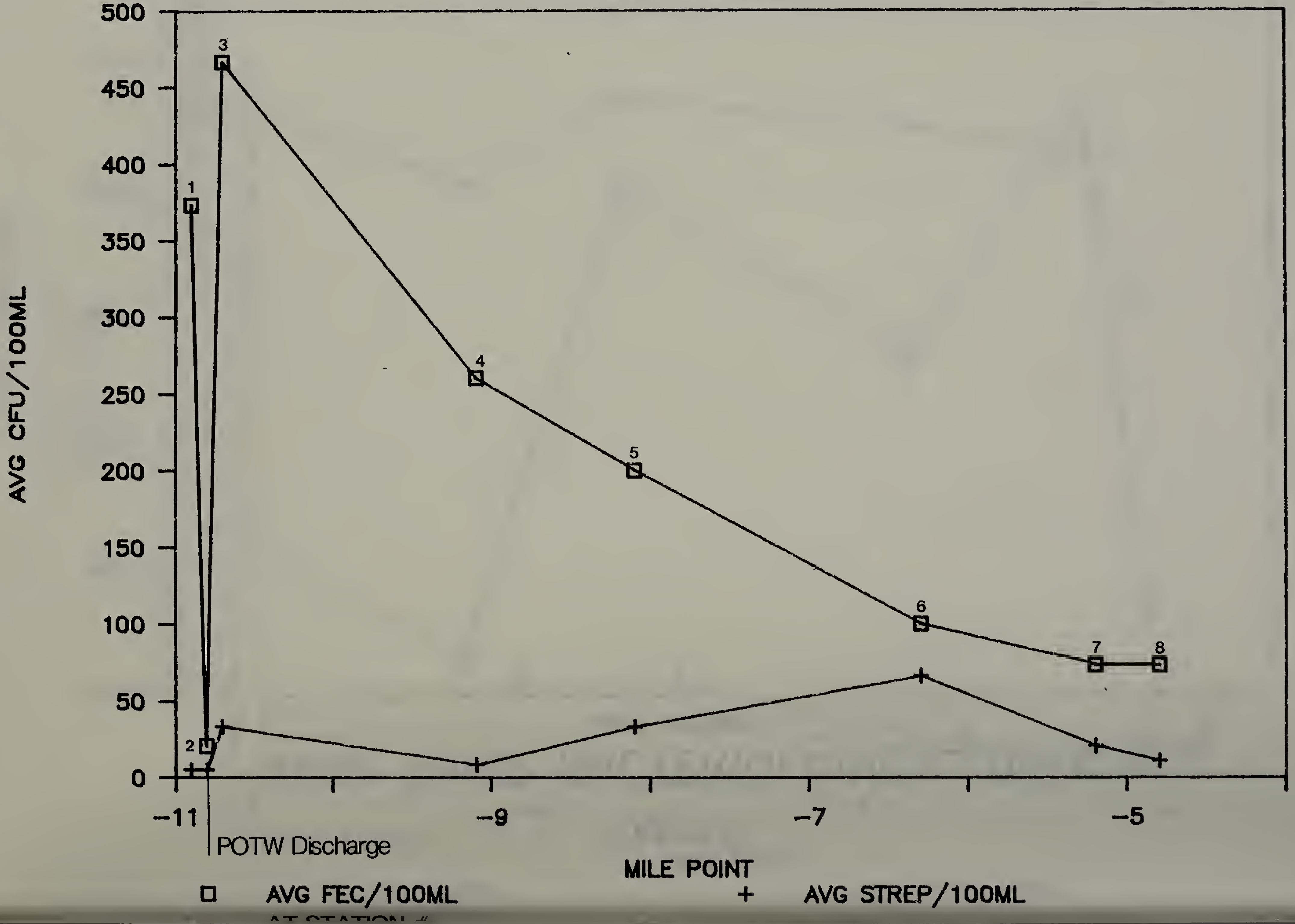


Figure 8

WARE RIVER BACTERIOLOGICAL DATA

JULY 29,1985

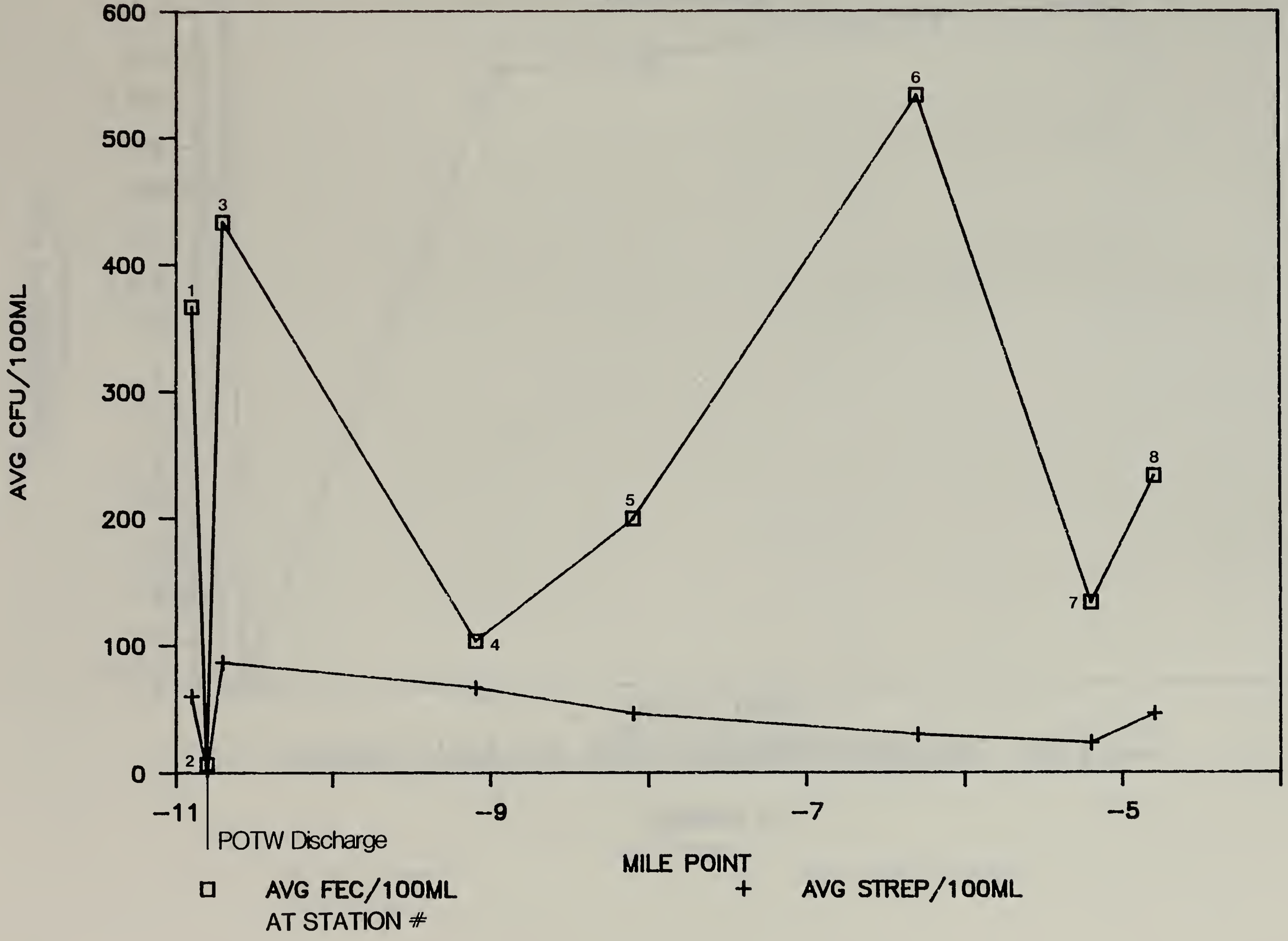


Figure 9

WARE RIVER BACTERIOLOGICAL DATA

JULY 31,1985

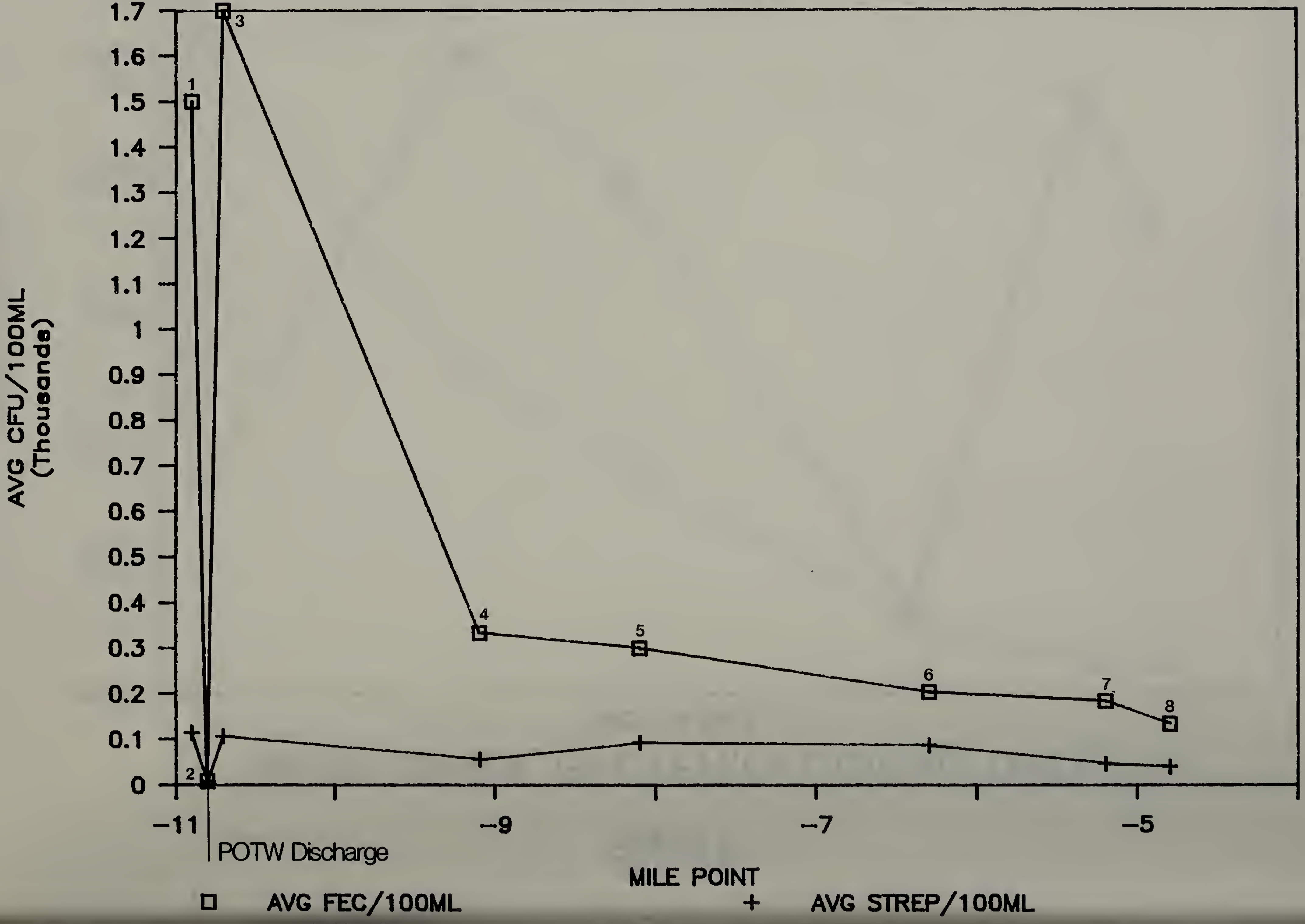


Figure 10

WARE RIVER BACTERIOLOGICAL DATA

AUGUST 05,1985

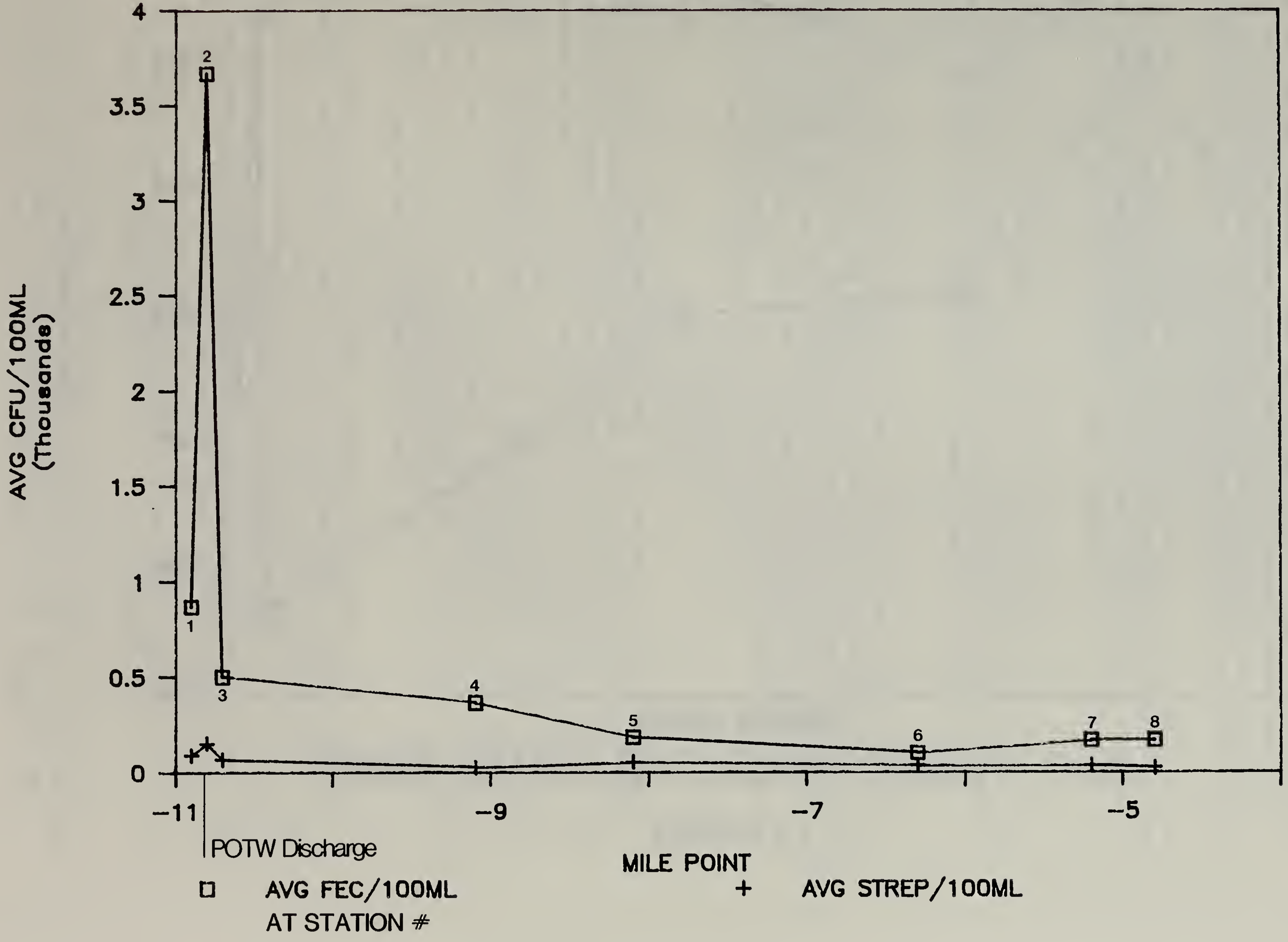
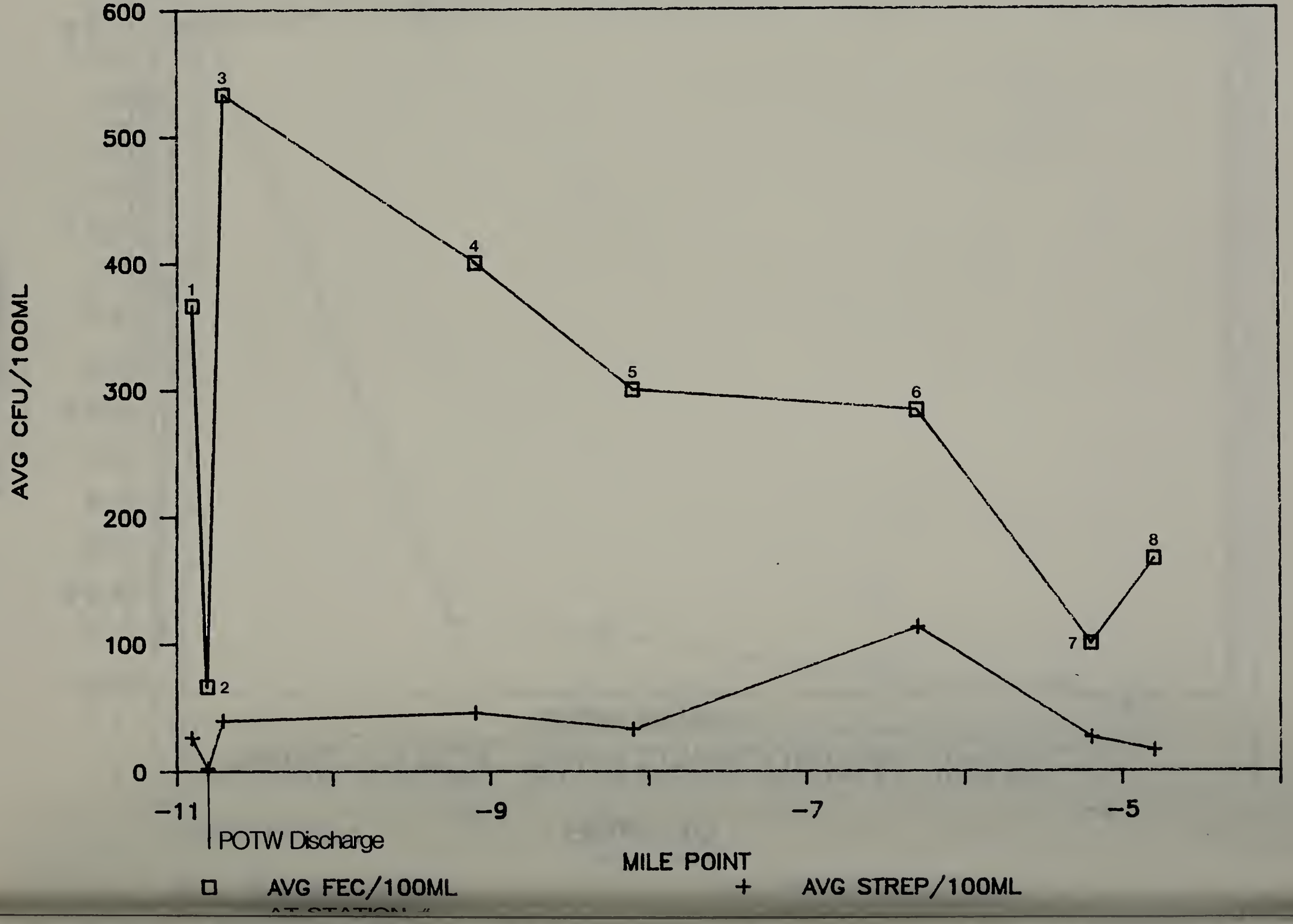


Figure 11

WARE RIVER BACTERIOLOGICAL DATA

AUGUST 07,1985



two and one-half miles. After decreasing again, the fecal coliform counts increased at the last station. The fecal streptococci decreased consistently over most of the sample stations except for the one furthest downstream (see Figure 8). Runoff which resulted from 0.72 inches of precipitation over the three previous days, probably was responsible for the erratic bacterial counts.

July 31 - The total chlorine residual was high at Station 2 (0.85 mg/l) with a mean fecal coliform count of 8/100 ml. The die-off curve again showed a steep drop over the first two miles and then a slower rate over the remaining stations. The fecal streptococci showed a slight decrease over the six miles (see Figure 9).

August 5 - The fecal coliform mean at Station 2 was 3666/100 ml with a total chlorine residual of 0.1 mg/l. The fecal coliform decreased steadily over the approximately four miles and then showed a slight increase over the remaining 1.5 miles. The fecal streptococci also decreased over this distance (see Figure 10).

August 7 - The fecal coliform bacteria showed a rapid decrease over the first two miles, leveled off and then showed a rapid decrease between Stations 6 and 7. At the last station there was an increase in fecal coliform bacteria. The fecal streptococci die-off did not follow the fecal coliform die-off. Instead the counts were fairly consistent over the first three miles, showed an increase at Station 6, and then decreased. A low chlorine residual was requested and was measured at 0.3 mg/l (see Figure 11).

Conclusions

The chlorine residual values that were found at Station 2 showed no apparent relationship with the bacterial counts found at this station. In some cases this is probably because the chlorine concentration was not held constant over the three-day period. It is also possible that the chlorination contact chamber, which is the old primary holding tank for the plant, may be operating at extremely long retention times so that the bacteria may be dying off while in this chamber. On July 24, DWPC requested no chlorine residual yet the fecal coliform counts were very low with a mean of approximately 20.8/100 ml. Because a question was raised if a toxicity problem may exist at the plant, a sample was collected from the chlorination chamber and tested with the Microtox™ Toxicity Analyzer. This sample was not found to be toxic.

Both upstream nonpoint sources of fecal contamination and the WWTP provided the population that was studied. While in the water they were affected by many influences which were not measured as part of this study. Factors which could affect die-off were not evaluated; only the overall die-off rate or growth rate were described. Sunlight was measured at each sampling station, but duration of sunlight and amount of unshaded area was not known. The amount of precipitation was also recorded. Under some rainfall conditions, increased runoff can occur which would affect the steady-state condition which was an assumption of this study. Increased flow can also affect time-of-travel, but upstream flow regulation caused much larger variations in the flow than precipitation did.

It is unlikely that the steady state regime was ever achieved. Problems occurred with rainstorms, cleaning out of holding tanks with chlorine compounds during "steady state" times, and apparent regulation of the flow upstream causing large diurnal variations in the flow (Gadoury et al., 1987).

RESULTS OF THE RIFFLE ZONE STUDY

Using flow information gathered by the Division of Water Pollution Control in 1972, 1973, 1980 (Kimball, 1972, 1973, 1980) the mixing zone below the Ware Wastewater Treatment Plant was calculated to be 2.3 miles (U.S. EPA, 1984).

Riffle zones are shallow, wide areas in a river where the turbulence of the water increases as it flows over the larger material (boulders, stones and gravel) which tend to congregrate on the bars that form the riffles (Hynes, 1970). They are followed by pools which are deeper and can have laminar flow. Riffle zones contribute to mixing. For this reason, the Ware WWTP discharges from a pipe located on the bank of the Ware River and empties into a riffle zone.

Although most fecal bacteria are killed by chlorination at the WWTP, some remain viable by chelating or adsorbing to organic particles in the waste effluent. These particles may be broken up by turbulence which could lead to a "release" of viable bacteria and an increase in bacterial counts. To examine if this phenomena might be occurring within the riffle zone, a grid of sampling stations was established, just below the WWTP discharge pipe. These stations extended in 50-foot increments downstream to 210 feet below the discharge pipe which was just outside of the riffle. Transects were also established across the river. This grid of sampling stations was chosen because it appeared to provide fairly good coverage of the riffle area.

Results and Discussion

Figures 12 to 16 represent the changes in the fecal coliform counts in the riffle zone and across the river. On July 24, 1985 sampling distances were only at 50, 100, and 150 feet. On July 29, July 31, August 5, and August 7 the sampling stations extended 210 feet below the discharge pipe. The stream flows recorded at Gibbs Crossing on these dates were:

DATE	FLOW(cfs)
July 24	36.9
July 29	73.0
July 31	36.9
August 5	52.7
August 7	36.9

On the days when the riffle zone was being sampled the viable fecal coliform bacteria contribution of the treatment plant was very small compared to that from upstream stations. Triplicates of the fecal coliform counts from above the Ware Wastewater Treatment Plant discharge (Station 1) and the WWTP discharge (Station 2) indicated that the upstream sources contributed more bacteria to the river than the WWTP on most of the days sampled. The arithmetic mean counts CFU/100 ml were:

DATE	STATION 1	STATION 2
July 24	373	21
July 29	367	7
July 31	1500	8

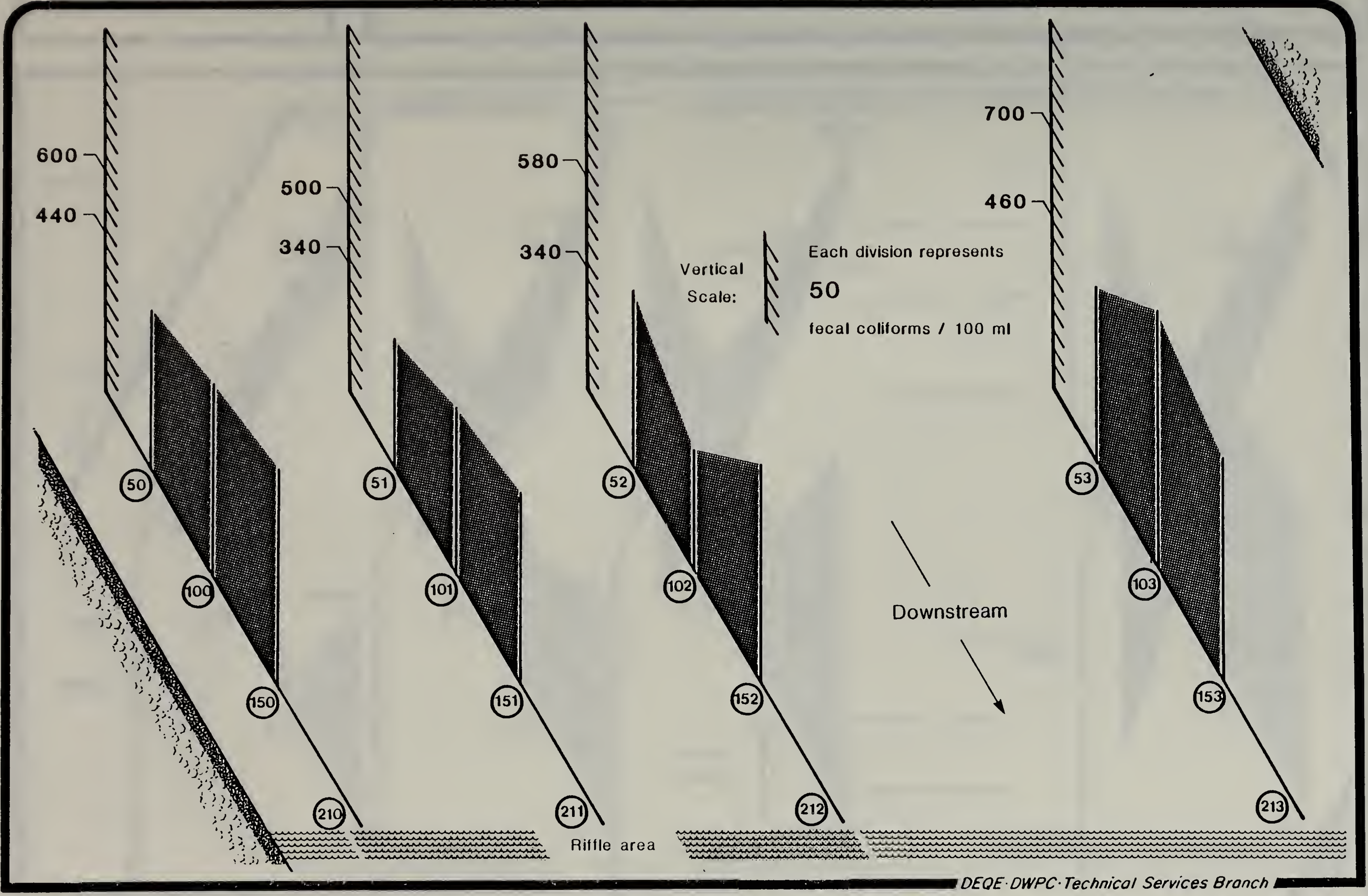
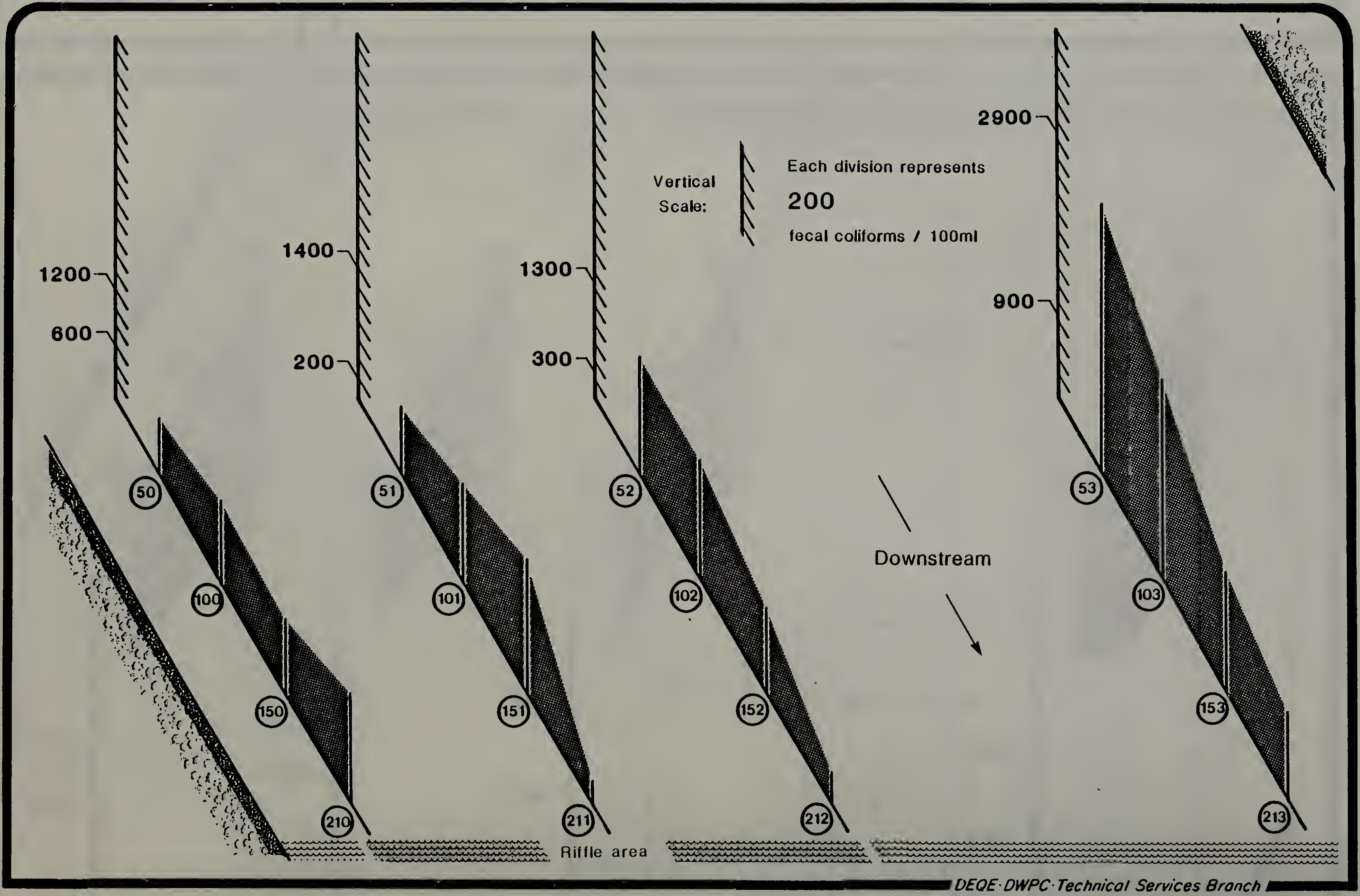


Figure 12

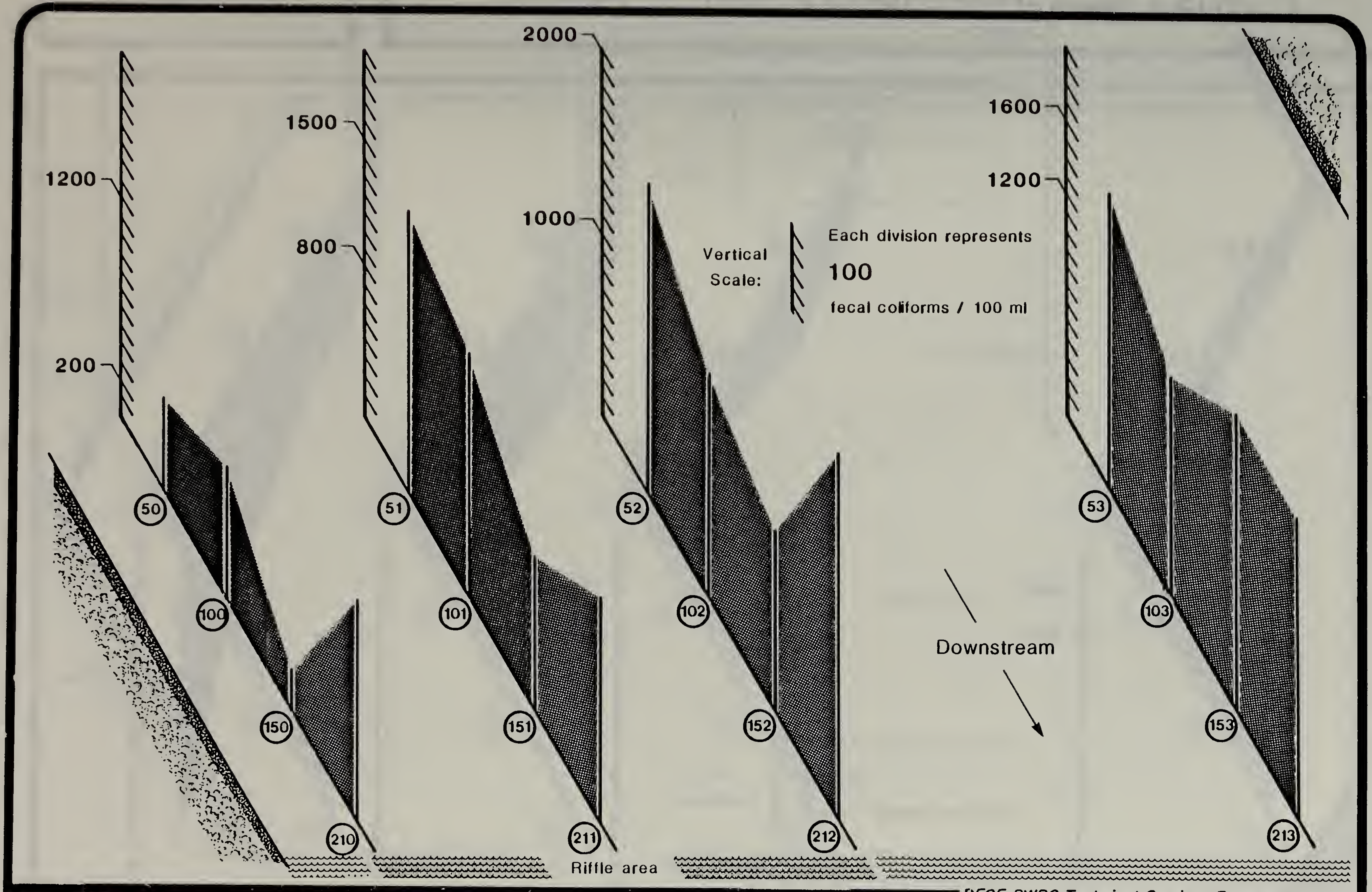
JULY 24, 1985 FECAL COLIFORM DENSITY



DEQE·DWPC·Technical Services Branch

Figure 13

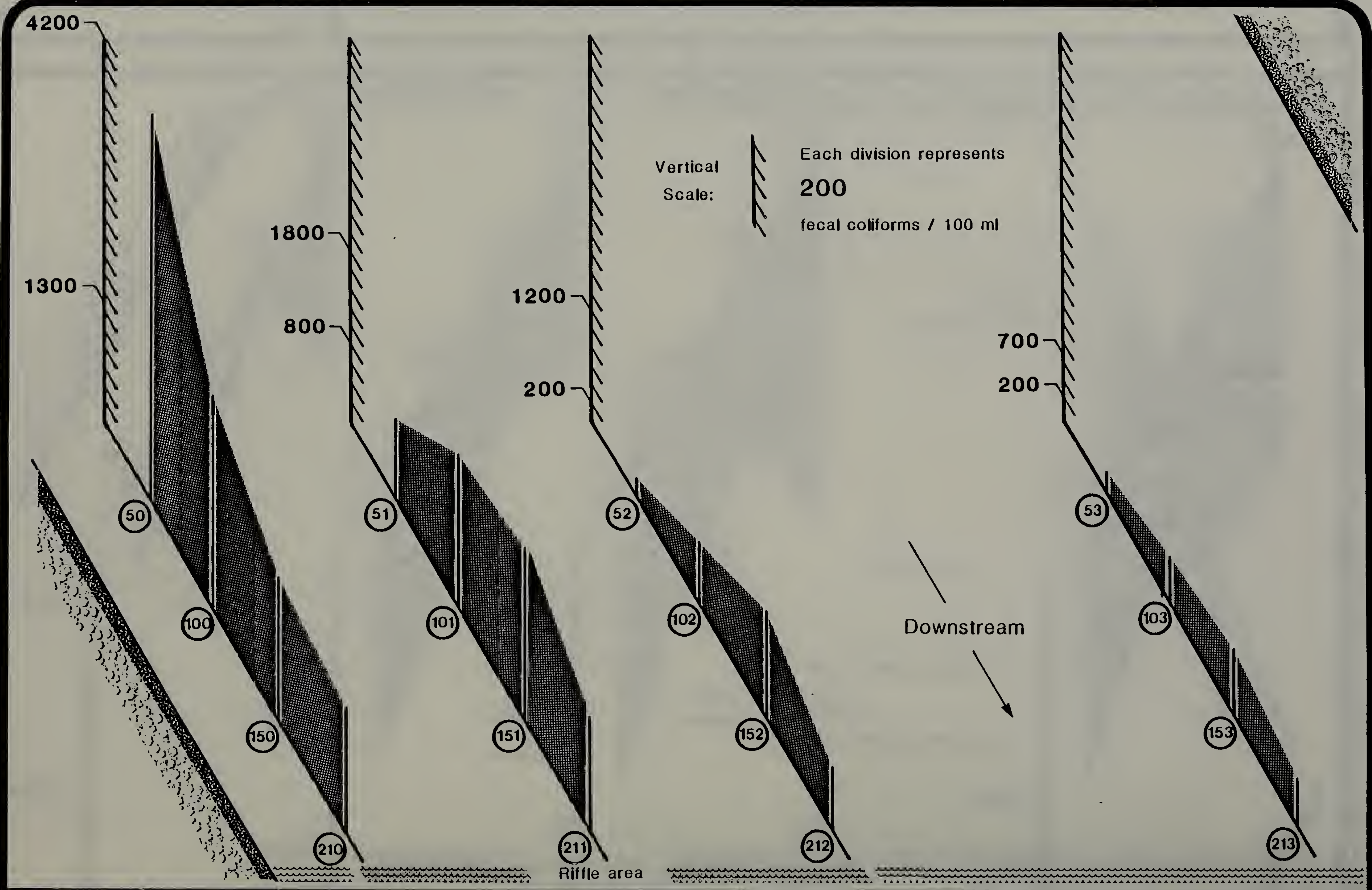
JULY 29, 1985 FECAL COLIFORM DENSITY



DEQE·DWPC·Technical Services Branch

Figure 14

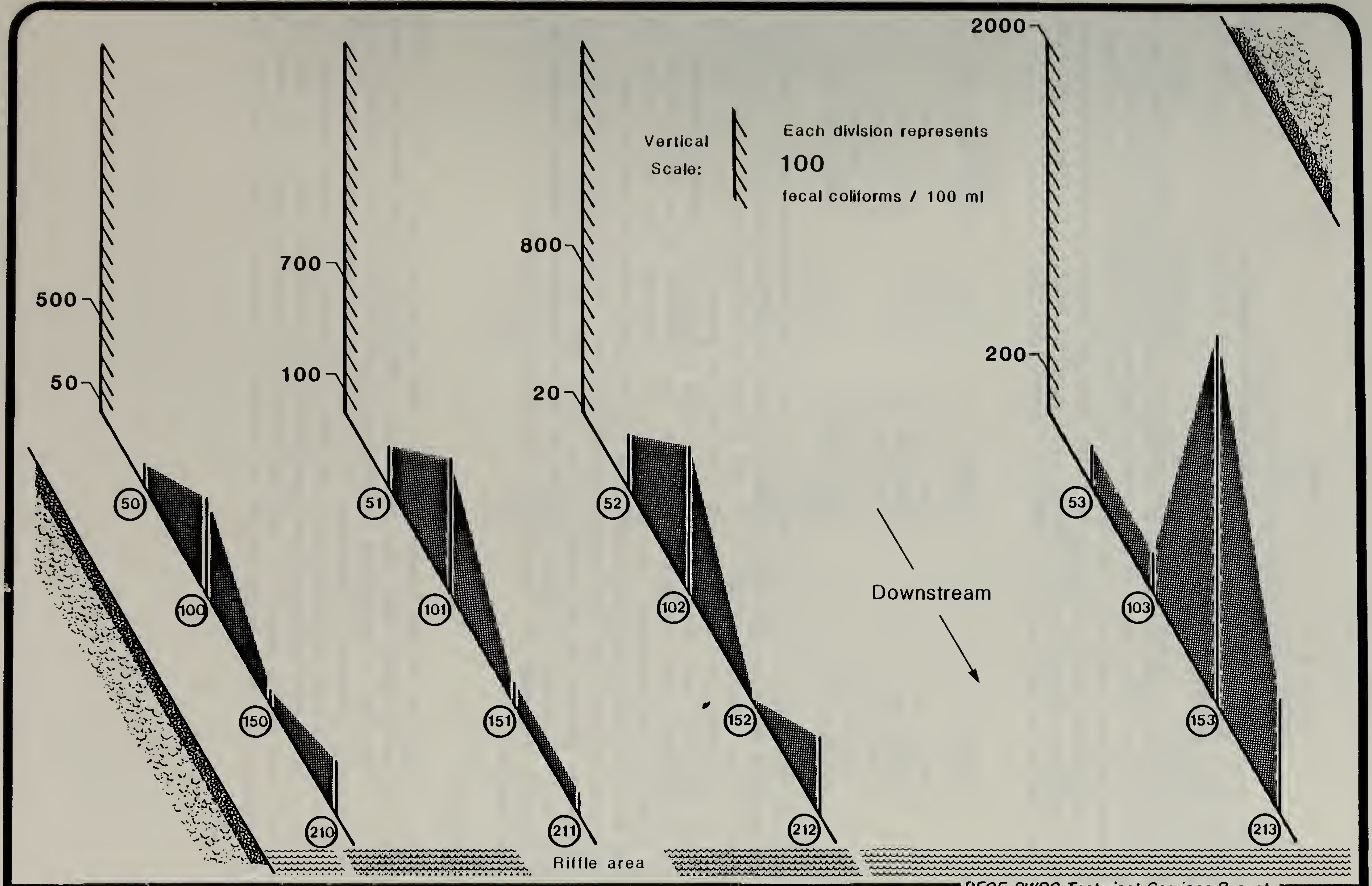
JULY 31, 1985 FECAL COLIFORM DENSITY



DEQE·DWPC·Technical Services Branch

Figure 15

AUGUST 5, 1985 FECAL COLIFORM DENSITY



DEQE·DWPC·Technical Services Branch

Figure 16

AUGUST 7, 1985 FECAL COLIFORM DENSITY

August 5	867	3666
August 7	367	67

Thus, the examination focussed on what happens to fecal particles in the riffle zone that have not been exposed to chlorine and are contributed by nonpoint sources rather than from the WWTP.

Some changes that occur in the fecal coliform counts over the riffle zone appear to be significant. To come to this conclusion standard deviations were first calculated on the fecal coliform samples collected at Station 1 just upstream from the treatment plant discharge. These samples were in triplicate and gave an indication of sample variability on each sampling day. The fecal coliform standard deviation and means for Station 1 were:

DATE	MEAN	STANDARD DEVIATION
July 24	373/100 ml	31/100 ml
July 29	367/100 ml	252/100 ml
July 31	1500/100 ml	200/100 ml
August 5	867/100 ml	723/100 ml
August 7	367/100 ml	58/100 ml

On July 24 (see Figure 12) the fecal coliform counts increased at all stations and in amounts greater than the variability determined at Station 1. On July 29 (Figure 13) and August 5 (Figure 15) the summation of stations 50, 51, 52, 53 (Table 7) in comparison to the summations of the groups 100, 101, 102, 103 and 150, 151, 152, 153 and 210, 211, 212, 213 show a decrease over this distance (i.e., from 5500/100 ml to 2600/100 ml and from 5400/100 ml to 3700/100 ml, respectively). By grouping the stations in this way the river was treated like a pipe and its flow like plug flow. The difference in these samples when grouped was greater than the sample variability as calculated for Station 1. This gives an indication of a decreasing count over this distance. On July 31 (Figure 14) and August 7 (Figure 16) there was an apparent increase over this same distance although the sample means showed great variability and thus were not conclusive.

Conclusions

It appears that the riffle zones do affect the bacterial counts, but in a manner that is not predictable from this data base. Reduction of the bacterial counts could be a function of predation by protozoa contributed by the treatment plant and thriving in nutrient rich waters (Kittrell and Furfari, 1963). The riffle zone also allow the break up of particles so that the bacterial counts increase. From these few sampling events and parameters, a prediction cannot be made of the impact the riffle zone will have on bacterial counts or how the presence of riffle zones might be related to the attainment of bacterial water quality standards.

TABLE 7

SUMMATION OF THE WARE RIVER
FECAL COLIFORM AND FECAL STREPTOCOCCI DATA

DATE	STATIONS	FECAL COLIFORM (CFU/100 ml)	FECAL STREPTOCOCCI (CFU/100 ml)
July 24, 1985	50-53	1720	230
	100-103	2020	300
	150-153	2280	280
July 29, 1985	50-53	5500	450
	100-103	5400	220
	150-153	4400	360
	210-213	2600	230
July 31, 1985	50-53	5300	310
	100-103	4400	330
	150-153	3600	220
	210-213	6000	320
August 5, 1985	50-53	5400	320
	100-103	5100	280
	150-153	5200	220
	210-213	3700	180
August 7, 1985	50-53	800	80
	100-103	2200	100
	150-153	2170	120
	210-213	1400	180

DISCUSSION

Die-Off/Regrowth Study

According to Deaner and Kerri (1965) some investigators have found the regrowth period to be approximately 9 to 15 hours after initial discharge into the receiving water. During this interval the coliform bacteria attain a peak density and then a rapid reduction follows. The data collected in this study did not conclusively show this effect even though the time-of-travel ranged from 12.0 to 52.8 hours (0.5 to 2.2 days):

DATE	TIME OF TRAVEL(days)
July 1	2.2
July 8	1.3
July 10	1.07
July 17	0.5
July 24	1.6
July 29	1.07
July 31	1.6
August 5	1.3
August 7	1.6

Deaner's study had conditions similar to this one with a chlorine residual of 2 ppm, a 45-minute contact period, water temperature which varied between 16-22°C and a 5.5 mile reach on the American River. Deaner concluded that the low turbidity in the river allowed high sunlight penetration which killed any bacteria in the water before regrowth could be initiated.

Shuval et al. (1973) also examined regrowth of fecal coliform that had been stressed by chlorination. They found accelerating growth after an interval of three days, resulting in a peak of 10^5 fecal coliform after starting with 10^2/100 ml. They also found an inverse correlation between coliform regrowth and residual chlorine concentrations. For example, 60% regrowth occurred with a chlorine residual of 0.35 ppm and 25% when the residual was 0.75 ppm. None of the travel times within this study extended for three days. The chlorination regime was similar to this one however.

In this study, the length of time available for regrowth of the fecal coliform bacteria may not have been sufficient for the stressed bacteria contributed by the Ware Treatment Plant to become viable. Since the treatment plant effluent typically had fecal coliform densities of 10^1 to 10^3 and human sewage typically exhibits 10^6 to 10^8 CFU/g of fecal coliform bacteria, there were potentially a great number of stressed bacteria contributed to the Ware River. The discharge from the Ware WWTP during two sampling periods 7/15-16, 1985 and 8/20-21, 1985 averaged 0.628 MGD (Massachusetts Division of Water Pollution Control 1986). Other factors such as water hardness and/or predation may be affecting these results, but they cannot be evaluated based on the information available.

It is not likely that a definitive study can be done to determine if fecal coliform bacteria regrow or multiply in the freshwater streams of Massachusetts because of the difficulties in finding a long enough stretch of river. This reach must not be below the input of a major tributary or other pollution sources. Thus, fecal coliform may not be monitored for a period long enough to learn if regrowth does occur. This has implications for water quality standards and classifications because if regrowth or multiplication does occur the areas downstream may be in violation of water quality standards, yet a true public health risk may not exist. A predictable fecal indicator to pathogen ratio is needed in order for water quality standards to be valid. Multiplication or regrowth of stressed fecal coliform bacteria would suggest that a different indicator organism should be used for environmental samples.

However, the question of multiplication of fecal coliforms in Massachusetts waters also must be addressed. In the Ware River samples, the majority of the viable bacteria at Station 3 were most likely contributed by non-chlorinated sources located upstream of the treatment plant. Over the distance of six miles the number of fecal coliform colony forming units typically decreased as a result of predation and sunlight. The most notable exception was Station 8 where often an increase was found. The overall KF rates were typically positive, indicating a decrease over time.

Riffle Zone Study

More information is necessary for a complete evaluation of the riffle zone including examination of the resuspension of bacteria, effect of variations in flow, determination of turbidity. Examination of the invertebrate population would also be useful in the analysis to aid in determining whether decreases in bacterial counts are likely to be the result of predation.

CONCLUSIONS

Die-Off/Regrowth Study

The fecal streptococci persisted slightly longer than the fecal coliform bacteria in the Ware River. The die-off rate of the fecal streptococci was half that of the fecal coliform bacteria. The fecal streptococci would be a better indicator of pathogens, such as the viruses that are known to persist longer in the environment.

Multiplication of the fecal coliform bacteria would have presented an additional problem in the interpretation of water quality. However, no significant evidence was seen of multiplication of unstressed fecal coliform bacteria. Bacteria, given proper nutrients and environmental conditions, can reproduce every 20 minutes. Given the nutrient input from the treatment plant and an upstream "seed" population, dramatic results could have occurred relatively rapidly over-riding the die-off phenomena, but this did not occur. The fecal streptococci also did not multiply in the segment of the Ware River that was studied.

Regrowth of the bacteria that were stressed by chlorination may have occurred. Station 8, the most downstream station, on six occasions showed a slight increase in fecal coliform counts over those at Station 7. Typically, the fecal streptococci counts did not increase within that same segment, giving some indication of regrowth of the fecal coliform bacteria. A longer time-of-travel is needed in order to prove this definitively.

Riffle Zone Study

On days of low flow (i.e., July 24, July 31, August 7) the summation of the bacterial counts increased over the riffle zone. On these days the flow was 36.9 cfs at approximately 10 a.m. On July 29 and August 5, with flows of 73 cfs and 52.7 cfs respectively, the summation of the bacterial counts decreased over the riffle zone.

No relationship was observed between changes in the bacterial counts over the riffle and the total chlorine residual measured at the Ware Wastewater Treatment Plant.

Riffle zones may play an important role in either the removal or the addition of viable bacterial colonies. More data would be needed to establish how the riffles work. Linear regressions could be done with turbidity and other parameters, as well as other bacterial indicators to further clarify these questions.

BIBLIOGRAPHY

American Public Health Assoc., 1985. Standard Methods for the Examination of Water and Wastewater 16th ed. American Public Health Assoc. Baltimore.

Camp Dresser and McKee, Inc., 1980. Waste Load Allocation Manual of Resource Analysis., Waltham, MA. 222 p.

Clausen, E.M., Green, B.L. and W. Litsky. 1977. "Fecal Streptococci: Indicators of Pollution." In: Bacterial Indicators/Health Hazards Associated with Water. A.W. Hoadley and B.J. Dutka. Eds. American Society for Testing and Materials. Philadelphia.

Deaner, D.G. and K. Kerri, 1965. "Regrowth of Fecal Coliform Bacteria." J. Am. Water Works Association. pp. 465-468.

Dufour, A.P. 1980. A 24-Hour Membrane Filter Procedure for Enumerating Enterococci. Presented at American Society for Microbiology Annual Meeting. Miami Beach, Florida.

Gadoury, R.A. and D.J. Kent, K.G. Ries III, H.L. White. 1987. Water Resources Data Massachusetts and Rhode Island Water Year 1985. U.S. Geological Survey Water Resources Division, Boston.

Hynes, H.B.N. 1970. The Ecology of Running Waters. University of Toronto Press. Toronto, Canada. 555 p.

Kenner, B., 1982. "Fecal Streptococcal Indicators" in: Indicators of Viruses in Water and Food. edit. G. Berg. Ann Arbor Science, Michigan.

Kimball, W. 1972, 1973, 1980. Unpublished Memoranda for the Record. Massachusetts Division of Water Pollution Control, Westborough, MA.

Kittrell, F.W. and S. Furfari. 1963. "Observations of Coliform Bacteria in Streams." Journal of the Water Pollution Control Federation. Vol. 35. 11-1361-1385.

Massachusetts Division of Water Polution Control. 1986. Chicopee River Basin. 1985. Ware and Chicopee Rivers Water Quality Survey. Massachusetts Division of Water Pollution Control, Technical Services Branch, Westborough. 38 p.

Shuvall, H., Cohen, J. and R. Kolodney. 1973. "Regrowth of Coliforms and Fecal Coliforms in Chlorinated Wastewater Effluents." Water Research. pp. 537-546.

U.S. Environmental Protection Agency. 1986. Ambient Water Quality Criteria for Bacteria. Office of Water Regulations and Standards. Washington, D.C.

U.S. Environmental Protection Agency. 1984. Technical Support Document for Water Quality Based Toxics Control. Draft. Office of Water. Washington, D.C.

APPENDIX

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A-6 Ware River Riffle Zone Bacterial Data - July 24, 1985

A-7 Ware River Bacterial Data - July 29, 1985

A-8 Ware River Riffle Zone Bacterial Data - July 29, 1985

A-9 Ware River Bacterial Data - July 31, 1985

A-10 Ware River Riffle Zone Bacterial Data - July 31, 1985

A-11 Ware River Bacterial Data - August 5, 1985

A-12 Ware River Riffle Zone Bacterial Data - August 5, 1985

A-13 Ware River Bacterial Data - August 7, 1985

A-14 Ware River Riffle Zone Bacterial Data - August 7, 1985

TABLE A-1

WARE RIVER BACTERIAL DATA

JULY 1, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)				FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\overline{X}^1$				$\overline{X}^1$				$\overline{X}^1$		
1	340	60	320	240	20	20	40	27	100	40	140	93	-	-
2	100	20	5	42	<10	<5	<5	3	<10	<5	<5	3	0.08	0.35
3	380	440	220	347	80	80	<5	54	180	40	20	80	-	-
4	100	120	160	127	40	20	20	27	40	740	20	267	-	-
5	200	160	320	227	<5	40	20	21	20	40	160	73	-	-
6	220	240	220	227	20	40	20	27	140	60	140	113	-	-
7	160	220	240	207	20	80	40	47	260	220	260	247	-	-
8	240	300	180	240	<5	20	20	14	40	80	220	113	-	-
Unnamed Tributary (5a)	20	-	-	-	140	-	-	-	2,200	-	-	-	-	-

$^1\overline{X}$ is the mean of the triplicate samples

TABLE A-2

WARE RIVER BACTERIAL DATA

JULY 8, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)				FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\overline{X}$[1]				$\overline{X}$[1]				$\overline{X}$[1]		
1	400	680	640	573	200	240	180	207	120	120	100	113	-	-
2	<10	<10	<10	5	<10	<10	<10	5	<10	<10	<10	5	0.5	1.0
3	880	800	560	747	240	460	240	313	100	3,600	120	1273	-	-
4	180	340	220	247	200	80	120	133	520	100	80	233	-	-
5	260	220	220	233	140	60	80	93	80	80	80	80	-	-
6	320	240	280	280	80	100	160	113	280	140	240	220	-	-
7	300	220	200	240	100	60	140	100	120	40	100	87	-	-
8	180	280	280	247	10	100	40	50	20	460	*TNTC	240	-	-
Unnamed Brook (5a)	<10	-	-	-	2,400	-	-	-	3,600	-	-	-	-	-

[1] $\overline{X}$ is the mean of the triplicate samples

TABLE A-3

WARE RIVER BACTERIAL DATA

JULY 10, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)				FREE Cl (mg/1)	TOTAL Cl (mg/1)
				$\overline{X}^1$				$\overline{X}^1$				$\overline{X}^1$		
1	380	460	300	380	200	180	280	220	240	160	200	200	-	-
2	14000	18000	16000	16000	9000	8000	10000	9000	10000	14000	12000	12000	~0.02	~0.02
3	400	600	460	487	320	260	280	287	240	340	160	247	-	-
4	700	900	800	800	400	380	500	427	440	300	480	407	-	-
5	960	780	680	807	480	420	400	433	400	360	440	400	-	-
6	200	300	220	240	160	280	100	180	80	100	120	100	-	-
7	200	260	200	220	60	60	160	93	80	80	40	67	-	-
8	240	260	320	273	200	240	220	220	140	100	140	127	-	-
Unnamed Brook (5a)	180	-	-	-	3,500	-	-	-	8,000	-	-	-	-	-

[1] $\overline{X}$ is the mean of the triplicate samples

TABLE A-4

WARE RIVER BACTERIAL DATA

JULY 17, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)	FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\overline{X}^1$				$\overline{X}^1$			
1	600	500	700	600	60	100	80	80		-	-
2	100	10	10	40	<5	<5	<5	3	Not sampled on this date	0.13	0.90
3	500	300	600	467	100	160	140	133		-	-
4	500	700	500	567	160	200	240	200		-	-
5	300	100	200	200	160	140	100	133		-	-
6	300	400	300	333	120	100	240	153		-	-
7	200	20	200	140	160	200	160	173		-	-
8	500	600	500	533	240	300	360	300		-	-
Unnamed Brook (5a)	10	-	-	-	3,000	-	-	-		-	-

[1] $\overline{X}$ is the mean of the triplicate samples

TABLE A-5

WARE RIVER BACTERIAL DATA

JULY 24, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)				FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\overline{X}$[1]				$\overline{X}$[1]				$\overline{X}$[1]		
1	380	340	400	373	<5	<5	10	5	20	20	40	27	-	-
2	20	40	<5	21	10	<5	<5	5	<5	<5	<5	3	0.06	0.05
3	400	520	480	467	40	20	40	33	100	160	100	120	-	-
4	280	240	260	260	<5	20	<5	8	<5	5	<5	3	-	-
5	240	220	140	200	20	40	40	33	<5	<5	60	22	-	-
6	120	60	120	100	100	40	60	67	80	<5	40	41	-	-
7	60	60	100	73	40	<5	20	21	<5	20	<5	8	-	-
8	20	80	120	73	<5	10	20	11	20	<5	<5	8	-	-
Unnamed Brook (5a)	20	-	-	-	100	-	-	-	80	-	-	-	-	-

[1] $\overline{X}$ is the mean of the triplicate samples

TABLE A-6

WARE RIVER RIFFLE ZONE BACTERIAL DATA

JULY 24, 1985

STATION	FECAL COLIFORM(CFU/100 ml)	FECAL STREPTOCOCCI(CFU/100 ml)	ENTEROCOCCI(CFU/100 ml)
50	440	40	60
51	340	50	40
52	480	60	60
53	460	80	40
100	540	120	180
101	440	80	80
102	340	40	80
103	700	60	100
150	600	40	<5
151	500	80	20
152	580	100	40
153	600	60	140

TABLE A-7

WARE RIVER BACTERIAL DATA

JULY 29, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)	FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\overline{X}^1$				$\overline{X}^1$			
1	600	400	100	367	40	80	60	60		-	-
2	10	5	5	7	<5	<5	<5	3	Not sampled on this date	0.05	0.55
3	500	500	300	433	80	100	80	87		-	-
4	100	10	200	103	60	100	40	67		-	-
5	100	200	300	200	40	60	60	53		-	-
6	300	600	700	533	10	20	60	30		-	-
7	200	100	100	133	20	10	40	23		-	-
8	200	100	400	233	20	40	80	47		-	-
Unnamed Brook (5a)	20	-	-	-	60	-	-	-		-	-

[1] $\overline{X}$ is the mean of the triplicate samples

TABLE A-8

WARE RIVER RIFFLE ZONE BACTERIAL DATA

JULY 29, 1985

STATION	FECAL COLIFORM (CFU/100 ml)	FECAL STREPTOCOCCI (CFU/100 ml)
50	600	70
51	700	120
52	1300	200
53	2900	60
100	900	40
101	1000	40
102	1300	80
103	2200	60
150	800	100
151	1400	60
152	900	80
153	1300	120
210	1200	60
211	200	10
212	300	20
213	900	140

TABLE A-9

WARE RIVER BACTERIAL DATA

JULY 31, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)	FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\overline{X}^1$				$\overline{X}^1$			
1	1300	1500	1700	1500	120	100	120	113	Not sampled on this date	-	-
2	10	10	5	8	<5	<5	20	8		0.55	0.85
3	1100	1600	2400	1700	100	120	100	107		-	-
4	300	600	100	333	20	140	10	57		-	-
5	200	400	300	300	80	100	100	93		-	-
6	400	10	200	203	80	60	120	87		-	-
7	150	300	100	183	60	60	20	47		-	-
8	100	200	100	133	20	60	40	40		-	-
Unnamed Brook (5a)	150	-	-	-	80	-	-	-		-	-

$^1\overline{X}$ is the mean of the triplicate samples

TABLE A-10

WARE RIVER RIFFLE ZONE BACTERIAL DATA

JULY 31, 1985

STATION	FECAL COLIFORM (CFU/100 ml)	FECAL STREPTOCOCCI (CFU/100 ml)
50	500	10
51	1500	60
52	1500	100
53	1600	140
100	700	20
101	1300	120
102	1200	90
103	1200	100
150	200	60
151	800	80
152	1000	20
153	1600	60
210	1200	80
211	1200	60
212	2000	80
213	1600	100

TABLE A-11

WARE RIVER BACTERIAL DATA

AUGUST 5, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)	FREE Cl (mg/1)	TOTAL Cl (mg/1)
				$\overline{X}^1$				$\overline{X}^1$			
1	400	500	1700	867	80	100	80	87		-	-
2	3000	4200	3800	3667	200	160	100	153	Not sampled on this date	0.07	~0.1
3	600	500	400	500	60	80	60	67		-	-
4	300	500	300	367	20	20	40	27		-	-
5	150	200	200	183	80	60	20	53		-	-
6	100	100	100	100	40	40	20	33		-	-
7	200	100	200	167	30	20	60	37		-	-
8	200	200	100	167	40	10	20	23		-	-
Unnamed Brook (5a)	10	-	-	-	400	-	-	-		-	-

[1] $\overline{X}$ is the mean of the triplicate samples

TABLE A-12

WARE RIVER RIFFLE ZONE BACTERIAL DATA

AUGUST 5, 1985

STATION	FECAL COLIFORM (CFU/100 ml)	FECAL STREPTOCOCCI (CFU/100 ml)
50	4200	140
51	800	40
52	200	80
53	200	60
100	2300	100
101	1600	80
102	700	60
103	500	40
150	1500	100
151	1800	60
152	1200	20
153	700	40
210	1300	40
211	1200	60
212	700	40
213	500	40

TABLE A-13

WARE RIVER BACTERIAL DATA

AUGUST 7, 1985

STATION	FECAL COLIFORM(CFU/100 ml)				FECAL STREPTOCOCCI(CFU/100 ml)				ENTEROCOCCI(CFU/100 ml)	FREE Cl (mg/l)	TOTAL Cl (mg/l)
				$\bar{X}^1$				$\bar{X}^1$			
1	300	400	400	367	40	20	20	27	Not sampled on this date	-	-
2	100	50	50	67	<5	<5	<5	3		0.15	0.30
3	200	500	900	533	60	20 →	40 →	40		-	-
4	300	500	400	400	20	40 →	80 →	47		-	-
5	100	400	400	300	60	20	20	33		-	-
6	400	50	300	283	160	100	80	113		-	-
7	100	100	100	100	20	40	20	27		-	-
8	100	100	300	167	20	10	20	17		-	-
Unnamed Brook (5a)	100	-	-	-	140	-	-	-		-	-

$^1\bar{X}$ is the mean of the triplicate samples

TABLE A-14

WARE RIVER RIFFLE ZONE BACTERIAL DATA

AUGUST 7, 1985

STATION	FECAL COLIFORM (CFU/100 ml)	FECAL STREPTOCOCCI (CFU/100 ml)
50	100	10
51	200	40
52	300	20
53	200	10
100	500	20
101	700	20
102	800	40
103	200	20
150	50	10
151	100	40
152	20	10
153	2000	60
210	300	40
211	100	80
212	400	20
213	600	40